Morphogens and blood-brain barrier function in health and disease

Nienke R Wevers¹ and Helga E de Vries^{2,*}

¹MIMETAS BV; Leiden, The Netherlands; ²Department of Molecular Cell Biology and Immunology; Neuroscience Campus Amsterdam; VU University Medical Center; Amsterdam, The Netherlands

Keywords: blood-brain barrier, brain diseases, morphogens, neurogenesis, neurovascular unit

Abbreviations: β -TrCP, β -transducin repeat containing protein; A β , amyloid β ; ABC, ATP-binding cassette; ACM, astrocyte conditioned medium; AD, Alzheimer's disease; AJ, adherens junction; ALS, amyotrophic lateral sclerosis; Ang, angiopoietin; Angptl, angiopoietin-like; APP/PS1, amyloid precursor protein/prenilin 1; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; BMVEC, brain microvascular endothelial cell; Br, barrier resistance; BSA-FITC, bovine serum albumin–fluorescein isothiocyanate; CCL2/MCP1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1; CNS, central nervous systems; Dvl, dishevelled; EAE, experimental autoimmune encephalomyelitis; EC, endothelial cell; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-related kinases; Fz, frizzled; Gli-1, GLI family zinc finger 1; GLUT, glucose transporter; GSK, glycogen synthase kinase; hCMC/D3, human cerebral microvascular endothelial cell line D3; hPSCs, human pluripotent stem cells; HUVEC, human umbilical vein endothelial cell; ICAM, intracellular adhesion molecule; IgG, immunoglobulin G; IL, interleukin; IP, intraperitoneal; IV, intravenous; JAM, junctional adhesion molecule; Lef, lymphoid enhancer factor; LRP, low density lipoprotein receptor-related protein; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MBEC4, mouse brain endothelial cell line 4; MRP, multidrug resistance protein; MS, multiple sclerosis; Na-F, sodium fluoride; NRP, neuropilin; NVU, neurovascular unit; OPC, oligodendrocyte precursor cell; P-gp, P-glycoprotein; PD, Parkinson's disease; PECAM, platelet endothelial cell adhesion molecule; PI3K, phosphoinositide 3-kinase; PLXN, plexin; PP2A, protein phosphatase 2A; PSGL, P-selectin glycoprotein ligand; Ptch, patched; qPCR, quantitative polymerase chain reaction; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RBE.4, rat brain microvessel endothelial (cell line) 4; RBP, retinol binding protein; RNS, reactive nitrogen species; ROS, reactive oxygen species; RT-qPCR, real time quantitative polymerase chain reaction; RXR, retinoid X receptor; Sema, semaphorin; Shh, sonic hedgehog; Smo, smoothened; SOX, sex-determining region Y-box; STRA6, membrane-bound protein stimulated by retinoic acid gene 6; TCF, T cell factor; TEER, transendothelial electrical resistance; TGF, transforming growth factor; Tie, endothelial tyrosine kinase; TJ, tight junction; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; Wnt, wingless-type murine-mammary-tumor virus integration site; ZO, zona occludens

The microvasculature of the brain forms a protective blood-brain barrier (BBB) that ensures a homeostatic environment for the central nervous system (CNS), which is essential for optimal brain functioning. The barrier properties of the brain endothelial cells are maintained by cells surrounding the capillaries, such as astrocytes and pericytes. Together with the endothelium and a basement membrane, these supporting cells form the neurovascular unit (NVU). Accumulating evidence indicates that the supporting cells of the NVU release a wide variety of soluble factors that induce and control barrier properties in a concentration-dependent manner. The current review provides a comprehensive overview of how such factors, called morphogens, influence BBB integrity and functioning. Since impaired BBB function is apparent in numerous CNS disorders and is often associated with disease severity, we also discuss the potential therapeutic value of these morphogens, as they may represent promising therapies for a wide variety of CNS disorders.

The Blood-Brain Barrier

Properties of the blood-brain barrier

The blood-brain barrier (BBB) protects the central nervous system (CNS) by creating a homeostatic environment through various mechanisms. The barrier restricts large polar substances and potentially neurotoxic compounds from the circulation, including drugs, from passively diffusing into the brain. In addition, more lipophilic compounds and metabolic toxins are actively removed from the brain by members of the ATP-binding cassette (ABC) transporter family, also known as ABC transporters.¹ An important ABC transporter is P-glycoprotein (P-gp), a member of the family of multidrug resistance proteins (MRPs) that actively drives cellular efflux of a large variety of xenobiotic compounds out of the BBB endothelial cells. Brain endothelial cells also express other ABC transporters, including other members of the MRP family and breast cancer resistance protein (BCRP). MRPs act as organic anion transporters, but can also efflux neutral organic drugs. BCRP extrudes a variety of therapeutic drugs, including cytostatics. The MRPs, including P-gp, and the BCRPs show large overlap in substrate specificity, which prevents numerous drugs from reaching their therapeutic target in the brain. This hampers efficient treatment of a number of

*Correspondence to: Helga E de Vries, Email: he.devries@vumc.nl
Submitted: 06/18/2015; Revised: 08/26/2015; Accepted: 08/30/2015
<http://dx.doi.org/10.1080/21688370.2015.1090524>

CNS disorders. Vice versa, brain endothelial cells have highly efficient and specific transporter systems to provide the brain with its essential nutrients.² Various essential metabolites – including hexoses, amino acids, monocarboxylic acids, purines, nucleosides, amines, and ions – selectively enter the brain to meet the high demands of the neurons. In particular, glucose transporter -1 (GLUT-1), which actively transports glucose into the brain, is highly expressed at BBB endothelial cells.

Passage of hydrophilic molecules and immune cells is restricted by the presence of tight junction (TJ) and adherens junction (AJ) protein complexes that seal the brain microvascular endothelial cells (BMVECs).^{3,4} In AJs,³ vascular endothelial cadherins (VE-cadherins) connect via homotypic interactions adjacent endothelial cells and are anchored into the cell cytoplasm by α , β , and gamma catenins. In addition, platelet endothelial cell adhesion molecule 1 (PECAM-1) has been shown to be involved in adherens junctions. Similar to VE-cadherin, PECAM-1 is also anchored into the cell cytoplasm by catenins. The observation that PECAM-1 knockout animals show no apparent vascular malformations indicates that the functions of PECAM-1 can be fully compensated by other molecules, likely VE-cadherin itself.⁵ Together, VE-cadherin, PECAM-1, and catenin form the essential contact of the vascular endothelial cells and enable the formation of tight junctions (see Fig. 1).

Tight junction complexes (TJs) consist of different sets of proteins.^{3,4,6} The transmembrane protein occludin was one of the first TJ proteins to be discovered. In addition, claudin 3, claudin 5 and possibly claudin 12 also belong to the TJ proteins, as do the junctional adhesion molecules (JAMs). Similar to the AJ

proteins, TJ proteins of adjacent endothelial cells connect to each other and interact with several cytoplasmic proteins, including zona occludens (ZO) proteins 1-3, that link the junction proteins with the endothelial cytoskeleton, further tightening the barrier (Fig. 1). In addition to their structural functions, AJ and TJ proteins are also involved in the regulation of intracellular signaling pathways that influence BBB integrity.⁷

Cells surrounding the brain microvasculature, such as astrocytes and pericytes, secrete factors that strengthen the junctions between the endothelial cells and thus induce the proper barrier function of the BBB. Astrocytes not only induce the upregulation of junctional proteins, but are also essential for the expression of BBB-specific transporters, including P-gp and GLUT-1. Vice versa, the BBB endothelial cells regulate growth and differentiation of associated astrocytes,⁴ illustrating the continuous communication in the neurovascular unit. Pericytes, small contractile cells surrounding the brain capillaries, were first reported in 1873, but their function in BBB development and maintenance remained undefined for a long time. It is now known that pericytes contribute to BBB integrity and function by releasing factors that strengthen the TJs. In addition, pericytes also express several TJ molecules and barrier-related transporters, including occludin, claudin-12, JAMs, ZO-1, ZO-2 and P-gp, MRP-1, and GLUT-1.⁸ Components that contribute to the development and maintenance of the BBB are together referred to as the neurovascular unit (NVU; see Fig. 2). The NVU comprises the brain microvascular endothelial cells, the basement membrane, and supporting cells, including astrocytes and pericytes, but also neurons, microglia, and oligodendrocytes.

Figure 1. Junctional proteins of the blood-brain barrier. In adherens junctions (AJs), transmembrane proteins vascular endothelial cadherin (VE-cadherin) and platelet endothelial cell adhesion molecule 1 (PECAM-1) from adjacent cells connect via homotypic interactions and are anchored into the cytoplasm of the endothelial cells by β -catenin. In tight junctions (TJs), junctional adhesion molecules (JAMs), occludins, and claudins 3, 5 and possibly 12 are found. Zona occludens proteins 1-3 (ZO1-3) anchor these TJ proteins into the cytoplasm of the endothelial cells. For both β -catenin and the ZO proteins, this anchoring is the result of interactions with other sets of proteins, including other catenins, cingulin, vinculin, and actin. For sake of simplicity, these proteins are not shown in this figure.

Figure 2. Components of the neurovascular unit. Cerebral blood vessels are made up of endothelial cells, which are embedded in a basement membrane. Supporting cells, such as astrocytes and pericytes, secrete factors that help develop and support the barrier function of the BBB.

The blood-brain barrier in disease state

Compromised BBB function has been shown in numerous diseases of the CNS, including neurodegenerative and neuro-inflammatory disorders. There are several mechanisms that may reduce the integrity of the BBB, including disruption of AJ and TJ complexes and changes in transport properties.⁹ Many of these permeability-inducing and inflammatory processes start with the entrance of immune cells into the brain and/or local inflammatory processes.

Entrance of immune cells

The brain's assumed "immune privilege" is compromised in many diseases of the CNS, resulting in extensive leukocyte infiltration.^{10,11} This process is initiated upon the attachment of leukocytes to the inflamed vascular endothelium of the brain. To date it is still debated if selectins play a role in CNS entry of leukocytes, but in general P-selectins and vascular cell adhesion molecule – 1 (VCAM-1) are expressed on the surface of inflamed endothelial cells and interact with their leukocytic ligands, P-selectin glycoprotein ligand 1 (PSGL-1), or very late antigen-4 (VLA-4) to capture the cell. Subsequently, the leukocyte may roll along the endothelial cells and chemokines on the endothelium may activate leukocytic integrins, which in turn interact with VCAM-1 and intercellular adhesion molecule 1 (ICAM-1) on the endothelium to create a firm adhesion. Next, the chemokines on the endothelium mediate leukocyte locomotion along the endothelium, also called crawling, after which the leukocyte may enter the brain either through an endothelial cell (*transcellular diapedesis*) or via interendothelial junctions (*paracellular diapedesis*). It is important to note that the process of leukocyte infiltration into the CNS is not yet fully understood and that we do not exclude the possibility that new studies will reveal other sets of proteins involved in the process. For a schematic representation of leukocyte infiltration in the CNS, see **Figure 3**.

Inflammatory mediators

Immune cells in the brain can secrete cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6). Several studies have shown that cytokines increase BBB permeability and transport of various compounds across the barrier.¹² The enhanced BBB permeability results either from the opening of TJs or from enhanced pinocytotic activity combined with the formation of transendothelial channels.¹³ In addition to cytokines, immune cells also produce a range of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), highly reactive molecules that are one electron short. Free radicals are produced during cellular processes and do not harm the cells if balanced out by antioxidants, which interact with them by donating an electron. However, in many CNS disorders, both an overproduction of free radicals as well as diminished antioxidant capacity are observed. The elevated free radicals induce DNA damage, protein collapse, enzyme failure, and lipid destruction¹⁴ in various cell types including neurons, glial cells, and brain endothelial cells, resulting in increased BBB permeability.¹⁵

Figure 3. Leukocyte infiltration into the CNS. Leukocyte migration into the CNS occurs through a multi-step process, which is initiated by leukocyte capture by the endothelium. Communication between chemokines and chemokine receptors causes activation of leukocytic integrins and enables their interaction with endothelial ICAM-1 and VCAM-1, creating a firm adhesion. After this adhesion is established, the leukocyte crawls along the endothelium – again mediated by chemokine expression – until it enters the brain either through an endothelial cell (*transcellular diapedesis*) or via an interendothelial junction (*paracellular diapedesis*). After entering the brain, the leukocyte can secrete various molecules – such as cytokines, free radicals, and matrix metalloproteinases – that weaken the tight junctions, change the BBB's transport properties or degrade the basement membrane, thus further reducing the integrity of the blood-brain barrier.

The entered immune cells may also secrete matrix metalloproteinases (MMPs), of which levels may be further elevated by the local cytokines and free radicals. MMPs are inactive enzyme precursors, which are activated when their prodomain is cleaved. Activated MMPs are able of cleaving all kinds of extracellular matrix proteins as well as TJ proteins, thereby reducing the integrity of the BBB.¹⁶ MMP9 was shown to be involved in BBB breakdown in various CNS disorders, including stroke, multiple sclerosis, and brain traumas.⁹

Diseases in which the BBB is compromised

Reduced BBB function has been shown in numerous brain diseases, including brain trauma, edema, stroke, epilepsy, and neuroinflammatory diseases such as multiple sclerosis. Multiple sclerosis (MS) is a progressive disease of the CNS characterized by profound BBB dysfunction, inflammation, immune cell entry, demyelination, and axonal loss. MS disease symptoms include both physical and psychological symptoms and although there are therapies available to slow down disease progression, to date no curative treatment is available.¹⁷

More recent studies have also shown BBB dysfunction in the most prevalent neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. Alzheimer's disease (AD) is characterized by impaired cognitive functions, such as memory, language, planning, and executive functioning. Two main pathologies are seen in the Alzheimer's brain: a) amyloid β ($A\beta$) depositions, also called plaques, and b) tau depositions, also called tangles. Although AD was first described over a century

ago, there are no therapies available to cure or slow down the disease.¹⁸

Parkinson's disease (PD) is characterized by various motor symptoms, including tremor, rigidity, and a shuffling gait, as well as non-motor symptoms, including sleep disturbances, depression, and autonomic dysfunction. The pathology seen in PD includes α -synuclein inclusions in neurons, called Lewy bodies, and a loss of dopaminergic neurons from the striatum. There are currently several symptomatic treatments available for PD, but no cure.¹⁹ BBB breakdown was also observed in the less prevalent disorder amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. This disease is characterized by rapidly worsening motor deficits caused by degeneration of upper and lower motor neurons. To date, no therapy is available to cure ALS and patients often die within 5 years after diagnosis.²⁰

For many of the above mentioned disorders, it is still unclear whether BBB breakdown is a primary event or a consequence of surrounding stress within the CNS, such as trauma, protein aggregation, etc. However, in almost all these disorders, disease severity is correlated to the extent of BBB disruption. For this reason, compounds that prevent or restrict BBB breakdown represent a promising therapy for most CNS disorders. However, in depth knowledge on which factors influence the integrity of the BBB is lacking.

In recent years, it has become apparent that a number of substances that is involved in neurogenesis, also influences angiogenesis and/or barrierogenesis of the vascular beds in the brain. The cell fate of brain endothelial cells may therefore be influenced by the exposure to such substances, called morphogens. This review is the first to provide a comprehensive overview of the various morphogens involved in neurogenesis and the development and integrity of the BBB and compares their expression in health and disease.

Morphogens

Morphogens are signaling factors that direct cell differentiation by providing positional information to developing tissues. Morphogens are often present in a gradient, and the predominant view is that the local concentration of the morphogen that a cell encounters determines a specific pattern of gene expression in that cell (see Fig. 4).²¹ Several morphogens that are involved in neurogenesis, also seem to play a role in BBB development and maintenance. The most important morphogens are listed in Table 1 and will be discussed below in detail.

Sonic hedgehog

Sonic hedgehog (Shh) is a morphogen encoded by the SHH gene and fulfills its function as a transcriptional regulator through interaction with a series of different receptors. Binding of Shh to its receptor Patched-1 (Ptch-1) removes the inhibition of another receptor, the G-protein coupled receptor Smoothened (Smo), which is essential for activation of the transcription factor Gli-1.²² Shh has an essential role in vertebrate organogenesis by regulating the patterning of the anterior-posterior limb axis, the

Figure 4. Schematic representation of morphogen function. Morphogens influence cell fate in a concentration-dependent manner. The cells furthest away (green) from the source (blue) are exposed to a low morphogen concentration, which causes them to develop into cell type C. Cells closer to the source (white) experience a higher concentration, causing them to develop into cell type B. The cells closest to the source (yellow) develop into yet another cell type, cell type A.

ventral somites, and also the CNS. In the developing embryo, Shh is produced by the notochord and induces the floor plate and the neural tube,²³ from which the CNS develops. Shh is also involved in the differentiation of various neuronal cell types as well as in axon guidance, meaning that Shh influences the direction of axonal growth cones.²⁴ In addition to its function in neurogenesis, recent studies also indicate a role for Shh in CNS angiogenesis/barrierogenesis.

Research has shown that while Shh is predominantly expressed in astrocytes, it is not found in BBB endothelial cells, which, in contrast to the astrocytes, express high levels of Ptch-1 and Smo receptors.²⁵ These observations indicate that Shh signaling functions as a means of communication between perivascular astrocytes and the endothelial cells of the BBB. Interestingly, Shh exerts potent barrier strengthening effects by affecting the regulation of various TJ proteins, including occludin, JAM-A, VE-cadherin, claudin-3 and claudin-5.^{25,26} Induction of occludin, claudin-5 and ZO-1 may be the result of hedgehog induced activation of transcription factors Gli-1 and sex-determining region Y-box-18 (SOX-18). Intraperitoneal (IP) injection of antagonist cyclopamine abolished the upregulation of junctional proteins and caused acute BBB disruption in mice, which supports Shh's barrier inducing properties *in vivo*.²⁶ In addition to its role in BBB integrity, Shh was also shown to be

Table 1. General and CNS-specific functions of morphogens

Morphogen	Functions
Sonic Hedgehog (Shh)	General: Essential inductive signal in vertebrate organogenesis. Regulates patterning of the anterior-posterior limb axis, the ventral somites, and the central nervous system (CNS). In CNS: Shh is produced by the notochord and induces the floor plate and the neural tube, ²³ from which the CNS develops. Shh is involved in the differentiation of various neuronal cell types as well as in axon guidance. ²⁴
Wnt/ β -catenin	General: Initially investigated for its role in carcinogenesis. This pathway is now known to control various processes in both vertebrate and invertebrate embryonic development, including cell fate specification, cell proliferation, cell migration, and body axis patterning. In CNS: Wnt/ β -catenin signaling induces neurogenesis in various CNS structures, including the spinal cord, hindbrain floor plate, ³⁴ midbrain, ³⁵ and telencephalon. ³⁶
Retinoic acid (RA)	General: Retinoic acid is a metabolite of vitamin A and is important during early embryonic development. Regulates organogenesis and patterning of the anterior-posterior axis. In CNS: Retinoic acid induces neuronal differentiation ⁴⁶ and axon guidance ⁴⁷ and is also involved in adult neurogenesis from the dentate gyrus. ⁴⁸
Transforming growth factor β (TGF- β)	General: TGF- β proteins are cytokines and regulate cell proliferation, differentiation, adhesion, migration, and the function of other growth factors. In CNS: The role of TGF- β in the CNS is less well understood than for the other morphogens. Members of the TGF- β family are involved in the switch of NPCs from neurogenesis to gliogenesis in the adult brain. ^{63,64} TGF- β is considered a prototypic inducer of epithelial-to-mesenchymal transition (EMT) ⁶² .
Bone morphogenetic proteins (BMP)	General: BMPs are cytokines belonging to the same TGF- β superfamily as the TGF- β subfamily described above and were first discovered for their role in osteogenesis. BMPs are important during embryonic development in embryonic patterning and skeleton formation. BMP dysregulation is involved in many disease processes, including carcinogenesis. BMP is also involved in the regulation of EMT. In CNS: During embryonic development, BMPs are involved in the formation of the neural tube and the spinal cord. ⁷⁸ Similar to TGF- β , BMPs inhibit

(Continued on next column)

Table 1. General and CNS-specific functions of morphogens (Continued)

Morphogen	Functions
	neurogenesis in the adult brain by promoting gliogenesis instead of neurogenesis. ^{63,64}
Angiopoietins (Ang)	General: Angiopoietins influence angiogenesis, the formation of blood vessels, and their stability. While Ang-1 and Ang-4 promote vessel stability, Ang-2 disrupts vascularization. In CNS: Ang-1 promotes neurogenesis in adults after cerebral ischemia. ⁹² Ang-2 regulates neurogenesis in the developing telencephalon as well as in adult neurogenesis. ⁹³
Semaphorins (Sema)	General: Semaphorins have many different functions, but were discovered for their role in axon guidance. Semaphorins play a role in the development of almost every organ. In CNS: Semaphorins have many roles in neurogenesis and especially axon guidance. In general, semaphorins repel axonal growth cones, ¹¹¹ but specific semaphorins attract the growth cones of specific neuronal subpopulations. ^{112,113}

involved in inducing CNS immune quiescence, one of the main functions of the BBB. Apical stimulation of the brain endothelial cells with players of the hedgehog pathway (hrShh, ACM, or purmorphamin treatment) significantly decreased ICAM-1 expression on BBB endothelial cells and the production of pro-inflammatory mediators involved in immune cell trafficking into the brain.²⁵

Hedgehog signaling is known to be upregulated in acute brain injury²⁷ and nerve injury.²⁸ In addition, hedgehog upregulation was shown in neuroinflammatory processes. In an inflammatory environment, BBB astrocytes increase Shh expression, while BBB endothelial cells showed higher Ptch-1 and Smo expression, indicating Shh pathway activation. It is proposed that Shh upregulation functions to promote BBB repair and counterbalance inflammatory events, aiming to prevent further damage.²⁵ Aberrant hedgehog signaling is seen in several CNS disorders. Reduced Shh levels were found in gray matter of Alzheimer's, Huntington's, and Parkinson's disease and in both gray and white matter in multiple sclerosis.²⁹ Many studies are currently focusing on Shh as a potential therapy for these diseases. In rat models of Parkinson's disease, for instance, lentivirus-mediated delivery of Shh to the striatum was shown to reduce degeneration of dopaminergic neurons and stimulate neuroregeneration.³⁰ As future studies will further investigate Shh signaling in disease, targeting this pathway may represent a possible therapy for BBB disruption.

Wnt/ β -catenin signaling

Proteins of the wingless-type murine-mammary-tumor virus integration site family (Wnt) were initially discovered for their role in carcinogenesis. Today the Wnt proteins are known to

control many different processes and take part in several Wnt pathways. Two non-canonical/ β -catenin independent pathways exist alongside one canonical/ β -catenin dependent pathway. In the canonical pathway, β -catenin regulates the transcription of other proteins, but only after nuclear translocation, a process for which Wnt proteins are required. Absence of Wnt proteins inhibits β -catenin's translocation as a result of the formation of a degradation complex that phosphorylates β -catenin at Ser45, Thr41, Ser37, and Ser33.³¹ β -catenin is then ubiquitinated by β -TrCP and degraded in the proteasome.³² Conversely, when Wnt proteins are present, they bind to the Frizzled-4 (Fz4) receptor on the BBB endothelial cells and Fz4 forms a complex with the co-receptor low density protein receptor-related protein 5/6 (LRP5/6). In turn, the cytoplasmic protein dishevelled (Dvl) becomes activated and the protein axin, which is the main component of the before mentioned degradation complex, is recruited to the Fz4-LRP5/6 receptor complex. This disrupts the degradation complex, resulting in stabilization of cytoplasmic β -catenin. After accumulation of β -catenin in the cytoplasm, the protein is translocated to the nucleus where it disrupts the transcriptional suppressor groucho from its interaction with lymphoid enhancer factor (Lef)/T cell factor (TCF) transcriptional activators, thereby enabling gene transcription.³³ The Wnt proteins control a wide variety of essential processes in both vertebrate and invertebrate embryonic development, including cell fate specification, cell proliferation, cell migration, and body axis patterning. The canonical/ β -catenin dependent Wnt pathway induces neurogenesis in various CNS structures, including the spinal cord, hindbrain floor plate,³⁴ midbrain,³⁵ and telencephalon.³⁶ In addition to its role in neurogenesis, the Wnt/ β -catenin dependent pathway is also implicated in CNS angiogenesis and barrierogenesis.

Through a series of experiments in transgenic mice expressing lacZ only in cells in which Wnt/ β -catenin signaling is active, Daneman et al. (2009) showed that Wnt/ β -catenin signaling is essential for CNS, but not non-CNS, angiogenesis. By analyzing tissue slides of E12.5 mice with anti-LacZ-antibody and a vascular marker, the authors showed that Wnt signaling is only active in angiogenesis in the CNS, and not in non-CNS tissues such as the liver, the heart, and the lungs. In addition, β -catenin deficient mice were used to show that inhibition of Wnt/ β -catenin signaling led to severe defects in CNS vasculature, while leaving the vasculature of non-CNS tissues intact. Although β -catenin is part of the AJs between BBB endothelial cells, the abnormal CNS vasculature in this experiment is likely not due to disruption of the AJs and TJs, because levels of occludin, claudin-5, ZO-1, and VE-cadherin remained unchanged. Lastly, this study indicated that Wnt/ β -catenin signaling regulates the expression of the BBB-specific glucose transporter GLUT-1.³⁷

In addition to the role of Wnt/ β -catenin in CNS angiogenesis, this pathway is also involved in the development of the BBB. A study using transgenic mice revealed that selective deletion of β -catenin in brain endothelium reduced expression of claudin-3, which coincided with increased vascular permeability, whereas animals with enhanced β -catenin transcriptional activity expressed higher levels of claudin-3. Additionally, activation of

β -catenin signaling using Wnt3a was able to induce barrier properties in mouse brain microvascular endothelial cells in culture. These findings indicate an important role for Wnt/ β -catenin signaling particularly in the regulation of TJ protein claudin-3, since expression levels and localization of claudin-5, ZO-1 and VE-cadherin remained unaffected.³⁸

Aberrant Wnt/ β -catenin signaling has been implicated in various CNS disorders. In AD brain, A β binds to the Fz receptor, thereby inhibiting Wnt/ β -catenin signaling.^{39,40} Wnt/ β -catenin signaling has also been shown to be essential in the survival of dopaminergic striatal neurons, the cells that become degenerated in Parkinson's disease. Wnt signaling was shown to have neuroprotective effects in 2 mouse models of PD.^{41,42} Moreover, elevated levels of GSK-3 β , an important player in Wnt/ β -catenin signaling, were found in the striatum of post-mortem PD brain tissue⁴³ and genetic polymorphisms in the gene for GSK-3 β were observed in PD patients,⁴⁴ indicating aberrant Wnt/ β -catenin signaling in neurodegenerative disorders. While normal activity of the Wnt/ β -catenin pathway promotes myelination and remyelination in the CNS, overactivation of the pathway inhibits these processes. A study investigating remyelination in wild-type and transgenic rodents showed that hyperactive Wnt/ β -catenin signaling inhibits myelination and remyelination, suggesting that aberrant Wnt/ β -catenin signaling may also be involved in human white matter disorders, such as MS.⁴⁵ As aberrant Wnt/ β -catenin signaling has been observed in many CNS disorders, therapies that alter this signaling may represent promising treatments.

Retinoic acid

Retinoic acid (RA) is a metabolite of retinol, also known as vitamin A. Retinol is a lipophilic molecule and is bound to retinol binding protein (RBP) when in circulation. This retinol-RBP complex can be taken up from the blood stream by cells via the membrane-bound protein stimulated by retinoic acid gene 6 (STRA6). By means of 2 sequential oxidation steps involving retinaldehyde dehydrogenase (RALDH) enzymes, retinol is oxidized to RA, allowing it to bind to its receptor, the retinoic acid receptor (RAR), which forms a heterodimer with the retinoid X receptor (RXR). The binding of RA to its receptors alters the transcription of various genes.

The diversity of functions of RA in vertebrate development have been known for many years. RA regulates organogenesis and patterning of the anterior-posterior axis. In addition, RA induces neuronal differentiation⁴⁶ and axon guidance⁴⁷ and is also involved in adult neurogenesis from the dentate gyrus.⁴⁸

Involvement of RA in blood-brain barrier formation was shown only recently. Through a series of in vitro experiments, we were able to show that in particular RA is needed for the induction of BBB properties in human brain endothelial cells. Treatment with RA increased the expression of TJ proteins and induced high transendothelial electrical resistance (TEER), while decreasing levels of the permeability-inducing factor VEGF-A (vascular endothelial growth factor A).⁴⁹ Effects were predominantly mediated by RAR β , the most important receptor in CNS specific RA signaling. In addition to a role for RAR β in barrier integrity, RAR β also plays a role in the development of the BBB.

RAR antagonist BMS493 was given *per os* to pregnant mice from gestational age 10.5 to 16.5. The embryos of BMS193-treated mice showed higher BBB permeability, decreased levels of RAR β gene-expression, and decreased gene-expression of ZO-1 and VE-cadherin, while gene-expression of occludin and claudin-5 remained unaffected.⁴⁹ Similar effects were described using brain microvascular endothelial cells derived from human pluripotent stem cells (hPSCs), indicating that RA is a potent stimulator of BBB function.⁵⁰

Several studies have shown aberrant RA signaling in the brains of AD patients. Their findings, including elevated levels of RALDH and lowered retinoid levels in serum and plasma, indicate dysfunctional RA signaling in AD.^{51,52} In addition, intraperitoneal RA injection in an established AD mouse model, APP/PS1 mice, resulted in decreased A β deposition and tau phosphorylation, less glia activation, attenuated neuronal degeneration, and improved spatial learning and memory.⁵³ Moreover, A β reduces RA synthesis in Tg2576 mice, another established AD mouse model. Treatment with RAR α agonists stimulated A β clearance, inhibited tau phosphorylation, and improved cognition.⁵⁴

Because retinol functions as a free radical scavenger, the molecule has often been proposed as a potential target in PD. However, a dysfunction in retinol or retinoic acid signaling in PD patients remains to be established.⁵⁵ In animal models of PD on the other hand, treatment with retinoic acid reduced degeneration of dopaminergic neurons⁵⁶ and alleviated motor symptoms.⁵⁷ Similar to PD, direct evidence for aberrant retinol/retinoic acid signaling has not been found in multiple sclerosis. Many recent studies, however, propose targeting the RA pathway in MS because of its barrier inducing properties, immune quiescence inducing properties, free radical scavenging properties, and anti-inflammatory properties.⁵⁸⁻⁶⁰

Transforming growth factor β

Members of the transforming growth factor β (TGF- β) subfamily are cytokines and exert their functions by binding to a membrane bound TGF- β type II receptor dimer. This type II receptor dimer recruits a TGF- β type I receptor dimer and a hetero-tetrameric complex is formed. The type II receptor phosphorylates serine residues of the type I receptor, which results in the activation of several pathways, including the Smad pathway and the mitogen-activated protein kinase (MAPK) pathway. Downstream proteins of these pathways then accumulate in the nucleus where they act as transcription factors, regulating gene expression.⁶¹ TGF- β is also involved in the process of epithelial-to-mesenchymal transition (EMT).⁶² This is a process in which fully differentiated epithelial cells lose many of their epithelial characteristics and adopt features typical of mesenchymal cells. EMT is involved in embryonic development, cancer progression, fibrotic disease, and tissue regeneration.

Proteins of the TGF- β subfamily regulate numerous processes, including cell proliferation, differentiation, adhesion, and migration as well as the expression of other proteins. Although the specific role of TGF- β in the CNS is not yet very well understood, it has been shown that members of the TGF- β subfamily

are involved in the switch from neurogenesis to gliogenesis that takes place in neuronal precursor cells (NPCs) in the adult brain.^{63,64} In addition to its established role in angiogenesis, recent studies also indicate a role for TGF- β in regulating BBB development and integrity.

TGF- β signaling is thought to play a critical role in vascular development and function⁶⁵ and has been listed as a compound protecting against neurodegeneration for many years.⁶⁶ More recently, a study was performed investigating the role of TGF- β 1 on the permeability of brain endothelial cells and the functional activity of P-gp. Treatment of endothelial cells with TGF- β 1 reduced permeability of the cells to fluorescent dyes in a dose-dependent manner and induced P-gp function.⁶⁷ Both effects were impaired in the presence of an anti-TGF- β 1 antibody.⁶⁸ A different study showed that in addition to pericytes, oligodendrocyte precursor cells (OPCs) also support BBB integrity via TGF- β signaling. Endothelial cells of the rat brain endothelial cell line RBE.4 showed significantly lower permeability to dextran after treatment with OPC conditioned medium. The lower permeability was accompanied by an upregulation of TJ proteins occludin, claudin-5, and ZO-1. These observations were not seen in RBE.4 cells treated with OPC conditioned medium containing TGF- β receptor inhibitor SB431542, indicating the process was mediated by TGF- β signaling. Transgenic mice with OPC-specific TGF- β 1 deficiency showed massive cerebral hemorrhage and leakage of the large serum protein immunoglobulin G (IgG) through the BBB and degradation of ZO-1. This study showed that OPC-derived TGF- β plays an important role in BBB integrity, both in vitro and in vivo. The authors propose that TGF- β increases BBB integrity through activation of the MAPK/ERK pathway.⁶⁹

TGF- β may also be involved in many CNS disorders. AD patients show increased TGF- β levels in the brain, but decreased levels in serum (similar to amyloid β in AD).^{70,71} In addition, reduced levels of TGF- β type II receptors are found in human AD brains. To investigate whether reduced TGF- β signaling led to Alzheimer-like symptoms, transgenic mice were used expressing a kinase-deficient form of TGF- β receptor II, resulting in inhibition of TGF- β signaling. The transgenic mice showed enhanced neurodegeneration in various areas of the brain. Subsequent experiments in these mice showed that decreased TGF- β signaling also increases cerebral accumulation of amyloid β . The authors propose that agonizing the TGF- β type II receptor may be beneficial in AD.⁷²

Aberrant TGF- β signaling was also found in PD. TGF- β 1 levels were significantly higher in the dopaminergic striatal regions and ventricular cerebrospinal fluid in PD patients than in controls.⁷³ Moreover, it has been shown that TGF- β 1 treatment (apical) has neuroprotective effects on neuron-glia cultures of 2 established PD mouse models.⁷⁴ MS patients show elevated serum levels of TGF- β 1, with even higher augmentation during relapses.⁷⁵ In the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), intravenous (IV) injection of TGF- β 1 ameliorated symptoms and clinical disease course in many studies.⁷⁶ The potent immunosuppressive and free radical scavenging effects of TGF- β make it a potential therapeutic agent in the treatment of many CNS disorders.

Bone morphogenetic proteins

The TGF- β superfamily also comprises the subfamily of bone morphogenetic proteins (BMPs). BMPs exist in several isoforms and are released by neuronal progenitor cells and glial cells. BMPs can bind to members of the bone morphogenetic protein receptor (BMPR) family, including type I receptors BMPR1A, BMPR1B, and type II receptor BMPR2. The process of ligand binding and receptor activation is similar to that of TGF- β . The ligand binds to the type II receptor after which a type I receptor is recruited and becomes phosphorylated, which results in the activation of several pathways, again including the Smad pathway.⁶¹ Although first described for their function in bone development, BMPs influence many processes, including axis patterning and skeleton formation during embryonic development, but also CNS development,⁷⁷ for instance in the formation of the neural tube and the spinal cord.⁷⁸ Similar to TGF- β , BMP family members are involved in the switch that takes place in the adult brain that causes NPCs to differentiate into glial cells instead of neurons.⁶³ TGF- β and BMPs are also both involved in the process of EMT. While TGF- β is considered a prototypic inducer of EMT, BMPs are involved in EMT regulation.⁶² In addition to all these various processes in which BMPs are involved, they also play a role in the formation and maintenance of the BBB.

Using BMP receptor 1A (BMPR1A) knock-out mice, glial signaling through BMPR1A was shown to be essential for the termination of CNS angiogenesis. While the number of cerebral blood vessels was similar in mutant and control mice at E17 and P0, mutant mice showed more cerebral blood vessels at P10. In vitro experiments showed that BMPR1A knockout astrocytes produce significantly higher levels of VEGF, a factor that is essential for angiogenesis during mouse embryonic development and of which the production decreases after birth in healthy circumstances. The persistent high levels of VEGF observed in the mutant mice after birth imply that angiogenesis is not terminated in absence of BMPR1A. This indicates that BMP signaling is essential for the termination of CNS angiogenesis.⁷⁹ The same study also investigated the role of BMP signaling in BBB development. Evans blue dye was injected into the tail vein of control and BMPR1A knock-down mice. Only in the mutant mice, leakage of the dye was observed in tissues surrounding cortical blood vessels at P10 and P20, indicating the lack of a functional BBB. Follow-up experiments using immunoglobulin at 10 months of age showed that BBB leakage in the mutants persisted into adulthood. The authors found that mutant mice showed decreased adhesive properties of astrocytes to BBB endothelial cells, which may explain the impaired BBB formation. The persistent high VEGF levels seen in the mutant can also explain BBB leakage, as VEGF induces vessel permeability. To our knowledge, mechanistic studies into the effects of BMP on BBB properties remain to be performed.⁷⁹

BMPs play a yet undefined role in several neurological disorders. For example, elevated levels of BMP6 were found in the hippocampus of human AD patients and APP transgenic mice, an established mouse model of AD. BMP6 staining was localized predominantly around A β plaques. Treatment of rat

neuronal progenitor cells with A β resulted in an upregulation of BMP6 both at the RNA and protein level. No significant differences were found in the mRNA levels of BMP2 and BMP7. The authors propose that upregulated BMP6 levels impair adult neurogenesis, as markers of neurogenesis were altered in the hippocampus of AD patients and APP mice compared to controls.⁸⁰ In addition to BMP6, BMP4 was also shown to be upregulated in AD animal models and was associated with reduced neurogenesis in the hippocampus.^{81,82} While elevated BMP levels thus seem harmful in chronic neurodegenerative processes such as AD, other studies have found neuroprotective functions for BMPs in acute CNS injury. Also, BMP7 has been shown to be neuroprotective against nigrostriatal toxicity in the 6-hydroxydopamine rat model of PD.⁸³ Although it has not previously been shown that BMP levels are aberrant in PD, BMPs -2, -4, -5, -6 and -7 are described to induce dopaminergic differentiation. For this reason, BMPs are investigated to be used to generate transplantable dopaminergic neurons for the treatment of PD.^{84,85} In MS, aberrant BMP signaling has been established since many years. Elevated BMP levels were found in human demyelinating MS lesions as well as in the EAE mouse model.^{86,87} Because BMP4 inhibits the production of oligodendrocytes, the myelin producing cells of the CNS, several studies are focusing on BMP4 inhibition to promote remyelination in MS and other demyelinating disorders.⁸⁸ At present, the effects of BMPs in CNS diseases are not yet fully understood and seem to differ between BMP family members and disease processes. However, in the future, targeting BMP signaling may represent a possible treatment for CNS diseases.

Angiopoietins

Angiopoietins are growth factors involved in the regulation of blood vessel formation and integrity. Currently, 3 different angiopoietins are known in humans, angiopoietin (Ang) -1, -2, and -4. Additionally, there are 7 known angiopoietin-like proteins. All angiopoietins exert their function by binding to the endothelial-specific receptor tyrosine kinase, Tie-2. Binding of Ang-1 to the receptor causes phosphorylation of Tie-2 and induces association of endothelial cells with supporting cells, thereby promoting vascular maturation.⁸⁹ Conversely, binding of Ang-2 does not cause phosphorylation of Tie-2. Ang-2 is a natural antagonist of Ang-1 and causes vascular remodeling and apoptosis.⁹⁰ Ang-4 was discovered more recently, and is the human ortholog of murine Ang-3. Both are agonists of the Tie-2 receptor.⁹¹ While the role of angiopoietins in angiogenesis has been established for a long time, we have only begun to discover their involvement in neurogenesis in the last few years. Ang-1 was shown to promote neurogenesis in adult brain after cerebral ischemia⁹² while Ang-2 is involved in the developing telencephalon as well as in adult neurogenesis.⁹³ Next to their role in angiogenesis and neurogenesis, the angiopoietins are also involved in regulation of BBB function.

Studies using transgenic mice or Ang-1 administration in vitro have shown that Ang-1 protects the peripheral vasculature of adult mice from leakage induced by VEGF or inflammatory

Table 2. Crosstalk between the morphogens involved in BBB integrity and other common pathways

Smad signaling	
TGF- β BMPs	TGF- β and the BMPs both exert their functions through Smad signaling. TGF- β signaling activates Smad2/3 and BMP signaling activates Smad1/5/8. Both types of Smad signaling are promoted by Smad4 and inhibited by Smad6/7. ¹²⁶
RA	Retinoic acid was shown to interfere with TGF- β /Smad signaling by functioning as a co-activator of the Smad pathway via a RAR-Smad3 interaction. ¹²⁷ Retinoic acid was shown to suppress BMP/Smad signaling through regulation of p-Smad1 stability. ¹²⁸
Wnt	Wnt and TGF- β /BMP/Smad signaling show high levels of crosstalk. The most common format occurs in the nucleus, where the Smad and Lef/ β -catenin co-complexes synergistically regulate a set of shared target genes. Moreover, additional interactions, including Smad7- β -catenin, are also seen in the cytoplasm. ¹²⁹
Shh	Shh and TGF- β /BMP/Smad signaling show high levels of crosstalk in various cell types, tissues, and biological processes. ¹²⁹
Ang	The interactions between angiopoietin signaling and the Smad pathway are not yet fully understood and appear to depend on cellular context. ¹³⁰⁻¹³²
Sema	The interactions between semaphorins and the Smad pathway have not yet been thoroughly studied. However, recent studies showed that NRP-1 is able to bind and activate the latent form of TGF- β 1 ¹³³ and that NRP-1 upregulates TGF- β /BMP/Smad signaling in myofibroblasts. ¹³⁴
MAPK signaling	
TGF- β BMPs	TGF- β and the BMPs can also signal through non-Smad pathways that include various branches of the MAPK pathway as well as PI3K-Akt pathways. ¹³⁵
RA	Retinoic acid induces MAPK/ERK signaling. ¹³⁶
Wnt	Extensive crosstalk was shown between Wnt/ β -catenin and the various branches of MAPK signaling. The relationship between the 2 depends on the cellular context. ^{137,138}
Shh	Sonic hedgehog was shown to activate MAPK/ERK and PI3K-Akt signaling in adult muscle cells. ¹³⁹ In many other cell types and tissues, a direct interaction has not been found, but the pathways share involvement in many biological processes. ¹⁴⁰
Ang	The crosstalk between angiopoietins and MAPK signaling is not yet fully understood and depends on the cellular context. ¹⁴¹⁻¹⁴⁴
Sema	Sema4D was shown to activate MAPK signaling. ¹⁴⁵ Sema7A enhancement of axon outgrowth required activation of MAPK pathways. ¹⁴⁶
PI3K-Akt signaling	
TGF- β BMPs	In addition to Smad-dependent signaling, TGF- β and the BMPs can also signal through non-Smad pathways that include various branches of the MAPK pathway as well as PI3K-Akt pathways. ¹³⁵

(Continued on next column)

Table 2. Crosstalk between the morphogens involved in BBB integrity and other common pathways (Continued)

PI3K-Akt signaling	
RA	Retinoic acid induces PI3K-Akt signaling. ^{147,148}
Wnt	Wnt signaling and PI3K signaling show crosstalk through GSK-3 β , a common downstream target of both pathways. ¹⁴⁹
Shh	Sonic hedgehog was shown to activate MAPK/ERK and PI3K-Akt signaling in adult muscle cells. ¹³⁹ Moreover, PI3K-Akt activation was shown to be essential for Shh signaling under various circumstances. ¹⁵⁰
Ang	Ang-1 can promote endothelial cell survival through the activation of the PI3K-Akt pathway. ¹⁵¹ Under normal circumstances, Ang-2 antagonizes Ang-1, but in the absence of Ang-1, Ang-2 can act as an agonist of the Tie-2 receptor and activate the PI3K-Akt pathway. ¹⁵²
Sema	Different semaphorins regulate PI3K-Akt signaling in distinct ways. ¹⁵³

agents.^{94,95} A different study showed that 2-4 hours after cerebral ischemia in mice, an acute induction of VEGF was seen in combination with a downregulation of Ang-1. These changes were accompanied by BBB leakage, indicating a role for both VEGF and Ang-1 in the regulation of BBB integrity. The same proteins also seem to be involved in neovascularization, as VEGF, VEGF receptors, Ang-1, and Tie-2 were upregulated at the boundaries of ischemic lesions 2-28 days after stroke.⁹⁶ One year later, a different study showed that while Ang-1 and Tie-2 were present in all cerebral vessels in healthy brain, increased Ang-2 combined with decreased Ang-1 and Tie-2 were found during BBB breakdown. In the days after the injury, Ang-2 levels decreased while Ang-1 levels increased and new blood vessels formed.⁹⁷ Many more studies have been performed showing similar results. The permeability-lowering effects of Ang-1 on the BBB are due to upregulation of various junctional proteins, including ZO-1, occludin, and VE-cadherin⁹⁸ and counteraction of the permeability enhancing VEGF.⁹⁹ Conversely, Ang-2 exerts its permeability inducing effects through downregulation of TJ proteins, including ZO-1 and claudin-5.¹⁰⁰ Although less is known about the effects of the angiopoietin-like (Angptl) proteins, some studies have investigated their effects on BBB integrity. Transgenic mice overexpressing Angptl-1 showed less BBB damage following cerebral ischemia compared to wild-type mice¹⁰¹ and Angptl-4 (IV) was shown to increase VE-cadherin and claudin-5 while counteracting VEGF signaling, thereby increasing BBB integrity, in a mouse model of ischemic stroke.¹⁰²

Aberrant angiopoietin signaling has been shown in various CNS disorders. Elevated levels of Ang-2 and VEGF were found in cerebral blood vessels of AD patients, resulting in impaired BBB integrity.¹⁰³ While these factors normally promote angiogenesis, there is a no increased vascularity in AD, probably because of the anti-angiogenic properties of A β .¹⁰³ A different study showed elevated serum levels of Ang-1 in AD patients. The cognitive performance of the subjects was evaluated using the mini-mental state examination and a correlation was found

between Ang-1 serum levels and cognitive performance in all tested patients and controls. The authors propose that elevated Ang-1 is an attempt of the body to encounter vascular remodeling and BBB leakage in AD.¹⁰⁴ In addition to Ang-1 and Ang-2, Ang-4 also seems to be involved in AD. Polymorphisms in the gene for Ang-4 are associated with a familial form of mixed AD and vascular dementia.¹⁰⁵ While there is currently no evidence for a link between the angiopoietins and PD, the angiopoietins are implicated in the animal model of multiple sclerosis. Intravenous injection of Ang-1 reduced BBB leakage and inflammatory cell infiltration into the brain and spinal cord of EAE rats.¹⁰⁶ As the angiopoietins play an important role in vessel formation and

integrity, modulation of their pathways represents a possible therapy to treat BBB abnormalities after stroke or in other CNS diseases.

Semaphorins

Vertebrates possess 20 different semaphorin (Sema) genes. Semaphorins are proteins with a large variety of functions. There are 7 classes of semaphorins, of which classes 3-7 are present in vertebrates. Class 3 semaphorins are secreted, while semaphorins from classes 4-7 are transmembrane proteins.^{107,108} Semaphorins can bind to 2 types of transmembrane receptors: plexins (PLXNs) and neuropilins (NRPs),^{109,110} depending on what class the semaphorin is in. The semaphorins were first discovered for their role in axon guidance.¹¹¹ In general, semaphorins repel axonal growth cones, but specific semaphorins attract the growth cones of specific neuronal subpopulations. Sema3C for instance, promotes the growth of cortical axons,¹¹² while Sema3F promotes the growth of olfactory bulb axons.¹¹³ In addition, semaphorins are also involved in the development of almost every organ, endothelial migration and vascular formation,¹¹⁴ and immune responses.¹¹⁵

Figure 5. The morphogens involved in BBB development and integrity influence signaling of common pathways. Morphogens involved in BBB integrity also influence common pathways involved in many biological processes throughout the body. These pathways include the Smad pathway (top left), the MAPK pathway (top right), and the PI3K-Akt pathways (bottom). These pathways are involved in development, cell proliferation, cell differentiation, cell survival, and apoptosis and proper functioning of these pathways is essential for healthy functioning of the body.

Table 3. Involvement of morphogens in BBB development and maintenance

Morphogen	Secreted by	Role in BBB development/maintenance
Sonic hedgehog	Astrocytes Pericytes	Shh signaling causes upregulation of junctional proteins occludin, JAM-A, VE-cadherin, claudin-3, claudin-5 ²⁵ , and ZO-1. ²⁶ In addition, activation of the hedgehog pathway also supports CNS immune quiescence. ²⁵
Wnt/ β -catenin	Astrocytes	Wnt/ β -catenin is essential for CNS, but not non-CNS, angiogenesis and regulates the expression of BBB specific glucose transporter GLUT-1. ³⁷ In addition, Wnt/ β -catenin signaling induces barrier properties in brain microvascular endothelial cells by upregulation of TJ protein claudin-3. ³⁸
Retinoic acid	Astrocytes	Treatment of human brain microvascular endothelial cells with retinoic acid results in increased TEER and Br. RA treatment increased expression of junctional proteins VE-cadherin, occludin, ZO-1, and claudin-5 while decreasing VEGF levels. ^{49,50} In addition, RA treatment induced an upregulation of various BBB-specific transporters, such as P-gp, BCRP, STRA6, and MRPs 1, 2, and 5. ⁵⁰
Transforming growth factor β	Astrocytes Pericytes OPCs	TGF- β 1 signaling reduces BBB permeability ⁶⁷⁻⁶⁹ through upregulation of junctional proteins occludin, claudin-5, and ZO-1. ⁶⁹ In addition, TGF- β 1 also induces upregulation of BBB specific transporter P-gp. ⁶⁸
Bone morphogenetic proteins	Astrocytes NPCs	BMP signaling through BMP receptor 1A (BMPRI1A) is essential for the termination of angiogenesis and the concomitant decrease of VEGF. ⁷⁹ Mice lacking BMPRI1A show impaired BBB development and decreased adhesive properties of astrocytes to BBB endothelial cells. ⁷⁹
Angiopoietins	Astrocytes Pericytes	Angiopoietin-1 (Ang-1) promotes BBB integrity by upregulation of various junctional proteins, including occludin, VE-cadherin, and ZO-1 ⁹⁸ while counteracting the effects of permeability-inducing VEGF. ⁹⁹ Conversely, Ang-2 impaired BBB integrity by downregulating TJ proteins, such as ZO-1 and claudin-5. ¹⁰⁰

(Continued on next column)

Table 3. Involvement of morphogens in BBB development and maintenance (Continued)

Morphogen	Secreted by	Role in BBB development/maintenance
Semaphorins	Astrocytes	Semaphorin 3A impairs BBB integrity through reorganization of AJ protein VE-cadherin in human umbilical vein endothelial cells (HUVECs) in cooperation with VEGF. This effect was not seen after treatment with Semaphorin 3B and Semaphorin 3F. ¹¹⁷ Semaphorin 4D compromises BBB integrity through downregulation of TJ protein claudin-5. ¹¹⁸

vascular permeability to fluorescent dextran. Semaphorin 3A reduced serine/threonine phosphatase activity of protein phosphatase 2A (PP2A), resulting in VE-cadherin serine phosphorylation and internalization. Semaphorin 3A thus destabilizes the BBB by interrupting the protective effect of PP2A on VE-cadherin.¹¹⁷ Similar to Semaphorin 3A, Semaphorin 4D also compromises BBB integrity. Brain microvascular endothelial cells showed reduced TEER after Semaphorin 4D exposure. The reduced BBB integrity following Semaphorin 4D treatment involved downregulation of TJ protein claudin-5. BBB integrity was restored within 24 hours after addition of an anti-Semaphorin 4D antibody. A similar effect was seen in EAE mice, the animal model of multiple sclerosis. While untreated EAE mice showed increased BBB permeability compared to healthy controls, EAE mice treated with anti-Semaphorin 4D (IP) showed a strong decrease in fibrinogen leakage, indicating anti-Semaphorin 4D therapy increases BBB integrity *in vivo*.¹¹⁸

Aberrant semaphorin signaling has been shown in several CNS disorders. Accumulation of an internalized form of Semaphorin 3A is associated with the degeneration of hippocampal neurons in Alzheimer's disease. The accumulation of Semaphorin 3A overlapped with the appearance of tau, one of AD's main pathologies, suggesting a link between the 2.¹¹⁹ A genome-wide association study found a significant association between AD and a PLXNA4 single nucleotide polymorphism located in a region encoding the Semaphorin 3A binding domain. In addition, it was shown that Semaphorin 3A/PLXNA4 signaling influences tau phosphorylation.¹²⁰ There is currently no evidence that semaphorin signaling is involved in PD, although there is controversy on whether polymorphisms in the gene for Semaphorin 5A are associated with the disease.¹²¹ Recent studies indicate a role for the so-called 'immune semaphorins' – Semaphorin 3A, 4A, 4D, 6D, and 7A – in MS and EAE.¹²² The previously described study on Semaphorin 4D found that this semaphorin impairs BBB integrity, activates microglia, and inhibits remyelination in EAE mice and suggests anti-Semaphorin 4D therapy for the treatment of white matter disorders.¹¹⁸

Pathway interactions

All previously discussed morphogens regulate CNS angiogenesis and barrierogenesis through different but not independent

pathways. Morphogens and their downstream targets often target other pathways including the Smad pathway, the MAPK pathway, and the phosphoinositide 3-kinase-Akt (PI3K-Akt) pathway (see Table 2 and Fig. 5). However, these pathways are not specific for brain and BBB-associated processes, but essential for many common processes throughout the body, including cell proliferation, cell differentiation, and cell survival. The high level of crosstalk between the BBB-influencing morphogens and these common signaling pathways suggests that altered levels of one morphogen and/or its signaling may have a large impact on consequent pathways throughout the body.

Conclusion and Future Perspectives

Over the last decades and especially in the last few years, many studies have been performed into the substances that influence BBB functioning. In the current review we have described the roles of several of these substances, called morphogens, in the development and maintenance of the BBB. Morphogens can influence blood-brain barrier development and integrity by altering the expression of junctional proteins such as VE-cadherin, catenin, occludin, claudin, and zona occludens proteins, or by altering the BBB's transport properties. Most morphogens – including sonic hedgehog, Wnt/ β -catenin, retinoic acid, transforming growth factor β , several bone morphogenetic proteins, and angiopoietin-1 and -4 – promote BBB integrity. Conversely, angiopoietin-2, and several members of the semaphorin family impair barrier integrity (for an overview, see Table 3 and Fig. 6).

Interestingly, all of these morphogens are also involved in neurogenesis, indicating a link between neurogenesis and barrierogenesis. The different morphogens show high levels of crosstalk with other pathways. Among these pathways are the Smad pathway, the MAPK pathway, and the PI3K-Akt pathway, which are all essential for many biological processes throughout the body.

According to the World Health Organization, CNS disorders will become the second most prevalent cause of death in the upcoming years. Especially Alzheimer's disease and the other dementias will become more prevalent as the population in Western countries continues to age. In the Netherlands, there are currently ~265,000 people diagnosed with AD. This number is expected to double within the next 30 years. In the US, the same trend is expected, resulting in an expected 14 million AD patients in 2050. The increase in AD patients will not only place a heavy burden on the patients and their environments, but will also pose a big socio-economic problem as health care costs will skyrocket. While Alzheimer's disease was discovered over a century ago, there are still no therapies that cure the disease or slow down disease progression. There are therapies available for other common CNS disorders such as Parkinson's disease and multiple sclerosis, but these are symptomatic treatments that do not prevent deterioration of the patient. Although for most CNS disorders the exact cause remains unknown, they share a common feature, as BBB breakdown is involved in all of them and is associated with disease progression and severity. The new insights into BBB strengthening and weakening morphogens may lead to advances in the treatment of many CNS disorders, including the most prevalent ones.

Figure 6. The influence of morphogens on junctional proteins and BBB integrity. Cells of the neurovascular unit – predominantly astrocytes (blue) and pericytes (green) – secrete morphogens that influence the expression of junctional proteins including occludin (purple), claudins (orange), JAMs (pink), PECAM-1 (green), VE-cadherin (blue) and the molecules that anchor them into the cytoplasm of the endothelial cells, such as zona occludens proteins (gray and blue) and β -catenins (pink). Signaling of the following pathways increases barrier integrity through upregulation of junctional proteins: Shh, Wnt/ β -catenin, retinoic acid, TGF- β , BMPs, and Angiopoietin-1. Conversely, Ang-2 and 2 members of the semaphorin family, Sema3A and Sema4D, have been shown to reduce the integrity of the BBB by downregulation of junctional proteins.

Activation of the barrier-promoting morphogens on the one hand, or inhibition of the barrier-impairing morphogens on the other hand, could prevent BBB breakdown or enable barrier repair. However, it must be taken into consideration that altering the signaling of just one of the discussed morphogens will not just alter BBB integrity, but also many other signaling pathways that are essential for healthy functioning of the entire body. For instance, altering morphogen signaling will influence Smad, MAPK, and PI3K-Akt signaling, which may lead to aberrant cell cycle regulation and carcinogenesis. A solution to these unwanted effects may lie in the use of drug delivery techniques that target the BBB specifically. One possible technique is to fuse the therapeutic drug to a *molecular Trojan horse*, which is a second peptide or antibody that binds a specific receptor on the BBB, for example the BBB transferrin or insulin receptor. Other options are viral and viral-based

vectors for BBB crossing and the use of artificial drug carriers, such as micelles, liposomes, and polymeric nanoparticles. Although great advances have been made in recent years, the field of BBB drug targeting is in its infancy and only very few current treatments make use of BBB drug delivery techniques.^{123,124}

Because many of the discussed morphogens – such as Wnt/ β -catenin, TGF- β , and the BMPs – are essential for a wide range of biological processes and the angiopoietins are essential for vessel development and integrity throughout the body, we propose that of these morphogens the semaphorins are the best option for treating BBB breakdown in CNS disorders. Recent studies have shown promising results regarding the use of anti-sema4D therapies. The US based company Vaccinex recently developed a monoclonal antibody that recognizes Sema4D with high affinity and blocks interaction between Sema4D and its receptors. The drug, called VX15/2503, was shown to ameliorate disease symptoms, increase BBB integrity, and improve remyelination in the mouse models of multiple sclerosis¹¹⁸ and Huntington's disease.¹²⁵ Last month, VX15/2503 successfully completed its phase

1 clinical trial in multiple sclerosis patients. Fifty adult MS patients received VX15/2503 intravenously in a single dose (1, 3, 6, 10, or 20 mg/kg) to assess the safety and tolerability of the drug. Detailed results will be published in the near future. Meanwhile, a phase 2 clinical trial of VX15/2503 in Huntington's disease is planned to start within the next few months.

While diseases of the central nervous system are becoming more prevalent, satisfactory treatments are not available in most cases. Impaired blood-brain barrier integrity is a common feature of these diseases and is correlated with disease progression and severity. Knowledge about the mechanisms involved in BBB integrity provides us with a better understanding of CNS disorders and may lead to advances in their treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Shen S, Zhang W. ABC transporters and drug efflux at the blood-brain barrier. *Rev. Neurosci.* 2010; 21(1):29-53; PMID:20458886
- Spector R. Nutrient transport systems in brain: 40 years of progress. *J. Neurochem.* 2009; 111(2):315-20; PMID:19686385; <http://dx.doi.org/10.1111/j.1471-4159.2009.06326.x>
- Wolburg H, Lippoldt A. Tight junctions of the blood-brain barrier: development, composition and regulation. *Vasc. Pharmacol.* 2002; 38(6):323-37; PMID:12529927; [http://dx.doi.org/10.1016/S1537-1891\(02\)00200-8](http://dx.doi.org/10.1016/S1537-1891(02)00200-8)
- Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* 2006; 7(1):41-53; PMID:16371949; <http://dx.doi.org/10.1038/nrn1824>
- Duncan GS, Andrew DP, Takimoto H, Kaufman SA, Yoshida H, Spellberg J, de la Pompa JL, Elia A, Wakeham A, Karan-Tamir B, et al. Genetic evidence for functional redundancy of Platelet/Endothelial cell adhesion molecule-1 (PECAM-1): CD31-deficient mice reveal PECAM-1-dependent and PECAM-1-independent functions. *J. Immunol.* 1999; 162(5):3022-30; PMID:10072554
- Liu W-Y, Wang Z-B, Zhang L-C, Wei X, Li L. Tight junction in blood-brain barrier: an overview of structure, regulation, and regulator substances. *CNS Neurosci. Ther.* 2012; 18(8):609-15; PMID:22686334; <http://dx.doi.org/10.1111/j.1755-5949.2012.00340.x>
- Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. *J. Cell Biol.* 2015; 209(4):493-506; PMID:26008742; <http://dx.doi.org/10.1083/jcb.201412147>
- Sá-Pereira I, Brites D, Brito MA. Neurovascular unit: a focus on pericytes. *Mol. Neurobiol.* 2012; 45(2):327-47; <http://dx.doi.org/10.1007/s12035-012-8244-2>
- Daneman R. The blood-brain barrier in health and disease. *Ann. Neurol.* 2012; 72(5):648-72; PMID:23280789; <http://dx.doi.org/10.1002/ana.23648>
- Engelhardt B. Immune cell entry into the central nervous system: involvement of adhesion molecules and chemokines. *J. Neurol. Sci.* 2008; 274(1-2):23-6; PMID:18573502; <http://dx.doi.org/10.1016/j.jns.2008.05.019>
- Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol.* 2012; 33(12):579-89; PMID:22926201; <http://dx.doi.org/10.1016/j.it.2012.07.004>
- De Vries HE, Blom-Roosemalen MCM, Oosten M van, de Boer AG, van Berkel TJ, Breimer DD, Kuiper J. The influence of cytokines on the integrity of the blood-brain barrier in vitro. *J. Neuroimmunol.* 1996; 64(1):37-43; PMID:8598388; [http://dx.doi.org/10.1016/0165-5728\(95\)00148-4](http://dx.doi.org/10.1016/0165-5728(95)00148-4)
- Juhler M, Blasberg RG, Fenstermacher JD, Patlak CS, Paulson OB. A spatial analysis of the blood-brain barrier damage in experimental allergic encephalomyelitis. *J. Cereb. Blood Flow Metab.* 1985; 5(4):545-53; PMID:3877064; <http://dx.doi.org/10.1038/jcbfm.1985.82>
- Lee D-H, Gold R, Linker RA. Mechanisms of Oxidative Damage in Multiple Sclerosis and Neurodegenerative Diseases: Therapeutic Modulation via Fumaric Acid Esters. *Int. J. Mol. Sci.* 2012; 13(9):11783-803; PMID:23109883; <http://dx.doi.org/10.3390/ijms130911783>
- Griot C, Vandeveld M, Richard A, Peterhans E, Stocker R. Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Radic. Res. Commun.* 1990; 11(4-5):181-93; PMID:1965721; <http://dx.doi.org/10.3109/10715769009088915>
- Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front. Neurol.* 2013; 4:32; PMID:23565108
- Lassmann H, van Horssen J, Mahad D. Progressive multiple sclerosis: pathology and pathogenesis. *Nat. Rev. Neurol.* 2012; 8(11):647-56; PMID:23007702; <http://dx.doi.org/10.1038/nrneurol.2012.168>
- Carreiras MC, Mendes E, Perry MJ, Francisco AP, Marco-Contelles J. The multifactorial nature of Alzheimer's disease for developing potential therapeutics. *Curr. Top. Med. Chem.* 2013; 13(15):1745-70; PMID:23931435; <http://dx.doi.org/10.2174/15680266113139990135>
- Ozawa Y. Neurodegenerative disease: Pieces of the Parkinson's puzzle. *Nat. Rev. Neurosci.* 2010; 11(12):787-787; PMID:21132875; <http://dx.doi.org/10.1038/nrn2945>
- Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 2011; 7(11):639-49; PMID:21989247; <http://dx.doi.org/10.1038/nrneurol.2011.153>
- Ashe HL, Briscoe J. The interpretation of morphogen gradients. *Development* 2006; 133(3):385-94; PMID:16410409; <http://dx.doi.org/10.1242/dev.02238>
- Østerlund T, Kogerman P. Hedgehog signalling: how to get from Smo to C and Gli. *Trends Cell Biol.* 2006; 16(4):176-80; <http://dx.doi.org/10.1016/j.tcb.2006.02.004>
- Patten I, Placzek M. The role of Sonic hedgehog in neural tube patterning. *Cell. Mol. Life Sci.* 2000; 57(12):1695-708; PMID:11130176; <http://dx.doi.org/10.1007/PL00000652>
- Charron F, Stein E, Jeong J, McMahon AP, Tessier-Lavigne M. The Morphogen Sonic Hedgehog Is an Axonal Chemoattractant that Collaborates with Netrin-1 in Midline Axon Guidance. *Cell* 2003; 113(1):11-23; PMID:12679031; [http://dx.doi.org/10.1016/S0092-8674\(03\)00199-5](http://dx.doi.org/10.1016/S0092-8674(03)00199-5)
- Alvarez JL, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, Terouz S, Sabbagh M, Wosik K, Bourbonniere L, Bernard M, et al. The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science* 2011; 334(6063):1727-31; PMID:22144466; <http://dx.doi.org/10.1126/science.1206936>
- Wang Y, Jin S, Sonobe Y, Cheng Y, Horiuchi H, Parajuli B, Kawanokuchi J, Mizuno T, Takeuchi H, Suzumura A. Interleukin-1 β induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *Kira J, ed. PLoS One* 2014; 9(10):e110024; PMID:25313834; <http://dx.doi.org/10.1371/journal.pone.0110024>
- Amankulor NM, Hambardzumyan D, Pyontek SM, Becher PJ, Joyce JA, Holland EC. Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. *J. Neurosci.* 2009; 29(33):10299-308; PMID:19692604; <http://dx.doi.org/10.1523/JNEUROSCI.2500-09.2009>
- Xu QG, Midha R, Martinez JA, Guo GF, Zochodne DW. Facilitated sprouting in a peripheral nerve injury. *Neuroscience* 2008; 152(4):877-87; PMID:18358630; <http://dx.doi.org/10.1016/j.neuroscience.2008.01.060>
- Mastroradi FG, daCruz LAG, Wang H, Boggs J, Moscarello MA. The amount of sonic hedgehog in multiple sclerosis white matter is decreased and cleavage to the signaling peptide is deficient. *Mult. Scler.* 2003; 9(4):362-71; PMID:12926841; <http://dx.doi.org/10.1191/135245803ms9240a>
- Zhang Y, Dong W, Guo S, Zhao S, He S, Zhang L, Tang Y, Wang H. Lentivirus-mediated delivery of sonic hedgehog into the striatum stimulates neuroregeneration in a rat model of Parkinson disease. *Neurol. Sci.* 2014; 35(12):1931-40; PMID:25030123; <http://dx.doi.org/10.1007/s10072-014-1866-6>

31. Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, Zhang Z, Lin X, He X. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 2002; 108(6):837-47; PMID:11955436; [http://dx.doi.org/10.1016/S0092-8674\(02\)00685-2](http://dx.doi.org/10.1016/S0092-8674(02)00685-2)
32. Maniatis T. A ubiquitin ligase complex essential for the NF-kappaB, Wnt/Wingless, and Hedgehog signaling pathways. *Genes Dev.* 1999; 13(5):505-10; PMID:10072378; <http://dx.doi.org/10.1101/gad.13.5.505>
33. Buscariet M, Stifani S. The "Marx" of Groucho on development and disease. *Trends Cell Biol.* 2007; 17(7):353-61; PMID:17643306; <http://dx.doi.org/10.1016/j.tcb.2007.07.002>
34. Joksimovic M, Patel M, Taketo MM, Johnson R, Awatramani R. Ectopic Wnt/beta-catenin signaling induces neurogenesis in the spinal cord and hindbrain floor plate. *PLoS One* 2012; 7(1):e30266; PMID:22271670; <http://dx.doi.org/10.1371/journal.pone.0030266>
35. Tang M, Villaseca JC, Luo SX, Guitarte C, Lei S, Miyamoto Y, Taketo MM, Arenas E, Huang EJ. Interactions of Wnt/beta-catenin signaling and sonic hedgehog regulate the neurogenesis of ventral mid-brain dopamine neurons. *J. Neurosci.* 2010; 30(27):9280-91; PMID:20610763; <http://dx.doi.org/10.1523/JNEUROSCI.0860-10.2010>
36. Gulacsi AA, Anderson SA. Beta-catenin-mediated Wnt signaling regulates neurogenesis in the ventral telencephalon. *Nat. Neurosci.* 2008; 11(12):1383-91; PMID:18997789; <http://dx.doi.org/10.1038/nn.2226>
37. Daneman R, Agalliu D, Zhou L, Kuhnert F, Kuo CJ, Barres BA. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106(2):641-6; PMID:19129494; <http://dx.doi.org/10.1073/pnas.0805165106>
38. Liebner S, Corada M, Bangso T, Babbage J, Taddei A, Czupalla CJ, Reis M, Felici A, Wolburg H, Frutiger M, et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *J. Cell Biol.* 2008; 183(3):409-17; PMID:18955553; <http://dx.doi.org/10.1083/jcb.200806024>
39. Inestrosa N, De Ferrari GV, Garrido JL, Alvarez A, Olivares GH, Barria MI, Bronfman M, Chacón MA. Wnt signaling involvement in beta-amyloid-dependent neurodegeneration. *Neurochem. Int.* 2002; 41(5):341-4; PMID:12176076; [http://dx.doi.org/10.1016/S0197-0186\(02\)00056-6](http://dx.doi.org/10.1016/S0197-0186(02)00056-6)
40. Inestrosa NC, Toledo EM. The role of Wnt signaling in neuronal dysfunction in Alzheimer's disease. *Mol. Neurodegener.* 2008; 3(9); PMID:18652670; <http://dx.doi.org/10.1186/1750-1326-3-9>
41. L'Episcopo F, Tirolo C, Testa N, Caniglia S, Morale MC, Cossetti C, D'Adamo P, Zardini E, Andreoni L, Ihekwa AE, et al. Reactive astrocytes and Wnt/beta-catenin signaling link nigrostriatal injury to repair in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Neurobiol. Dis.* 2011; 41(2):508-27; <http://dx.doi.org/10.1016/j.nbd.2010.10.023>
42. L'Episcopo F, Tirolo C, Caniglia S, Testa N, Morale MC, Serapide MF, Pluchino S, Marchetti B. Targeting Wnt signaling at the neuroimmune interface for dopaminergic neuroprotection/repair in Parkinson's disease. *J. Mol. Cell Biol.* 2014; 6(1):13-26; <http://dx.doi.org/10.1093/jmcb/mjt053>
43. Duka T, Duka V, Joyce JN, Sidhu A. Alpha-Synuclein contributes to GSK-3beta-catalyzed Tau phosphorylation in Parkinson's disease models. *FASEB J.* 2009; 23(9):2820-30; PMID:19369384; <http://dx.doi.org/10.1096/fj.08-120410>
44. Kwok JBJ, Hallupp M, Loy CT, Chan DK, Woo J, Mellick GD, Buchanan DD, Silburn PA, Halliday GM, Schofield PR. GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. *Ann. Neurol.* 2005; 58(6):829-39; PMID:16315267; <http://dx.doi.org/10.1002/ana.20691>
45. Fancy SPJ, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Dev.* 2009; 23(13):1571-85; PMID:19515974; <http://dx.doi.org/10.1101/gad.1806309>
46. Yu S, Levi L, Siegel R, Noy N. Retinoic acid induces neurogenesis by activating both retinoic acid receptors (RARs) and peroxisome proliferator-activated receptor beta (PPAR-beta). *J. Biol. Chem.* 2012; 287(50):42195-205; PMID:23105114; <http://dx.doi.org/10.1074/jbc.M112.410381>
47. Dmetrichuk JM, Carlone RL, Spencer GE. Retinoic acid induces neurite outgrowth and growth cone turning in invertebrate neurons. *Dev. Biol.* 2006; 294(1):39-49; PMID:16626686; <http://dx.doi.org/10.1016/j.ydbio.2006.02.018>
48. Jacobs S, Lie DC, DeCicco KL, Shi Y, DeLuca LM, Gage FH, Evans RM. Retinoic acid is required early during adult neurogenesis in the dentate gyrus. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103(10):3902-7; PMID:16505366; <http://dx.doi.org/10.1073/pnas.0511294103>
49. Mizze MR, Wooldrik D, Lakeman KAM, van het Hof B, Drexhage JA, Geerts D, Bugiani M, Aronica E, Mebius RE, Prat A, et al. Retinoic acid induces blood-brain barrier development. *J. Neurosci.* 2013; 33(4):1660-71; PMID:23345238; <http://dx.doi.org/10.1523/JNEUROSCI.1338-12.2013>
50. Lippmann ES, Al-Ahmad A, Azarin SM, Palecek SP, Shusta EV. A retinoic acid-enhanced, multicellular human blood-brain barrier model derived from stem cell sources. *Sci. Rep.* 2014; 4:4160; PMID:24561821; <http://dx.doi.org/10.1038/srep04160>
51. Jiménez-Jiménez FJ, Molina JA, de Bustos F, Ortí-Pareja M, Benito-León J, Tallón-Barranco A, Gasalla T, Porta J, Arenas J. Serum levels of beta-carotene, alpha-carotene and vitamin A in patients with Alzheimer's disease. *Eur. J. Neurol.* 1999; 6(4):495-7; <http://dx.doi.org/10.1046/j.1468-1331.1999.640495.x>
52. Connor MJ, Sidell N. Retinoic acid synthesis in normal and Alzheimer diseased brain and human neural cells. *Mol. Chem. Neuropathol.* 1997; 30(3):239-52; PMID:9165489; <http://dx.doi.org/10.1007/BF02815101>
53. Ding Y, Qiao A, Wang Z, Goodwin JS, Lee ES, Block ML, Allsbrook M, McDonald MP, Fan GH. Retinoic acid attenuates beta-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model. *J. Neurosci.* 2008; 28(45):11622-34; PMID:18987198; <http://dx.doi.org/10.1523/JNEUROSCI.3153-08.2008>
54. Goncalves MB, Clarke E, Hobbs C, Malmqvist T, Deacon R, Jack J, Corcoran JP. Amyloid beta inhibits retinoic acid synthesis exacerbating Alzheimer disease pathology which can be attenuated by a retinoic acid receptor alpha agonist. *Eur. J. Neurosci.* 2013; 37(7):1182-92; PMID:23379615; <http://dx.doi.org/10.1111/efn.12142>
55. Takeda A, Nyssen OP, Syed A, Jansen E, Bueno-de-Mesquita B, Gallo V. Vitamin A and carotenoids and the risk of Parkinson's disease: a systematic review and meta-analysis. *Neuroepidemiology* 2014; 42(1):25-38; PMID:24356061; <http://dx.doi.org/10.1159/000355849>
56. Yin L-H, Shen H, Diaz-Ruiz O, Bäckman CM, Bae E, Yu SJ, Wang Y. Early post-treatment with 9-cis retinoic acid reduces neurodegeneration of dopaminergic neurons in a rat model of Parkinson's disease. *BMC Neurosci.* 2012; 13(1):120; PMID:23040108; <http://dx.doi.org/10.1186/1471-2202-13-120>
57. Ulusoy GK, Celik T, Kayir H, Gürsoy M, Isik AT, Uzbay TI. Effects of pioglitazone and retinoic acid in a rotenone model of Parkinson's disease. *Brain Res. Bull.* 2011; 85(6):380-4; PMID:21600965; <http://dx.doi.org/10.1016/j.brainresbull.2011.05.001>
58. Mizze MR, Nijland PG, van der Pol SMA, Drexhage JA, van Het Hof B, Mebius R, van der Valk P, van Horssen J, Reijerkerk A, de Vries HE. Astrocyte-derived retinoic acid: a novel regulator of blood-brain barrier function in multiple sclerosis. *Acta Neuropathol.* 2014; 128(5):691-703; PMID:25149081; <http://dx.doi.org/10.1007/s00401-014-1335-6>
59. Abrahi Froushani SM, Delirez N, Hobbenaghi R, Mosayebi G. Synergistic effects of atorvastatin and all-trans retinoic acid in ameliorating animal model of multiple sclerosis. *Immunol. Invest.* 2014; 43(1):54-68; PMID:24063549; <http://dx.doi.org/10.3109/08820139.2013.825269>
60. Fragoso YD, Stoney PN, McCaffery PJ. The evidence for a beneficial role of vitamin A in multiple sclerosis. *CNS Drugs* 2014; 28(4):291-9; PMID:24557746; <http://dx.doi.org/10.1007/s40263-014-0148-4>
61. Massagué J. TGF-beta signal transduction. *Annu. Rev. Biochem.* 1998; 67:753-91; <http://dx.doi.org/10.1146/annurev.biochem.67.1.753>
62. McCormack N, O'Dea S. Regulation of epithelial to mesenchymal transition by bone morphogenetic proteins. *Cell. Signal.* 2013; 25(12):2856-62; PMID:24044921; <http://dx.doi.org/10.1016/j.cellsig.2013.09.012>
63. Mattar P, Cayouette M. Temporal control of neural progenitors: TGF-beta switches the clock forward. *Neuron* 2014; 84(5):885-8; PMID:25475182; <http://dx.doi.org/10.1016/j.neuron.2014.11.013>
64. Stipursky J, Francis D, Dezonne RS, Bérnago de Araújo AP, Souza L, Moraes CA, Alcantara Gomes FC. TGF-beta1 promotes cerebral cortex radial glia-astrocyte differentiation in vivo. *Front. Cell. Neurosci.* 2014; 8:393; PMID:25484855; <http://dx.doi.org/10.3389/fncl.2014.00393>
65. Perrella MA, Jain MK, Lee ME. Role of TGF-beta in vascular development and vascular reactivity. *Miner. Electrolyte Metab.* 1998; 24(2-3):136-43; PMID:9525696; <http://dx.doi.org/10.1159/000057361>
66. Flanders KC, Ren RF, Lippa CF. Transforming growth factor-betas in neurodegenerative disease. *Prog. Neurobiol.* 1998; 54(1):71-85; PMID:9460794; [http://dx.doi.org/10.1016/S0304-0082\(97\)00066-X](http://dx.doi.org/10.1016/S0304-0082(97)00066-X)
67. Dohgu S, Yamauchi A, Takata F, Naito M, Tsuruo T, Higuchi S, Sawada Y, Kataoka Y. Transforming Growth Factor-1 Upregulates the Tight Junction and P-glycoprotein of Brain Microvascular Endothelial Cells. *Cell. Mol. Neurobiol.* 2004; 24(3):491-497; PMID:15206827; <http://dx.doi.org/10.1023/B:CEMN.0000022776.47302.ee>
68. Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, Tsuruo T, Sawada Y, Niwa M, Kataoka Y. Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-beta production. *Brain Res.* 2005; 1038(2):208-15; PMID:15757636; <http://dx.doi.org/10.1016/j.brainres.2005.01.027>
69. Seo JH, Maki T, Maeda M, Miyamoto N, Liang AC, Hayakawa K, Pham LD, Suwa F, Taguchi A, Matsuyama T, et al. Oligodendrocyte precursor cells support blood-brain barrier integrity via TGF-beta signaling. *PLoS One* 2014; 9(7):e103174; PMID:25078775; <http://dx.doi.org/10.1371/journal.pone.0103174>
70. Flanders KC, Lippa CF, Smith TW, Pollen DA, Sporn MB. Altered expression of transforming growth factor-beta in Alzheimer's disease. *Neurology* 1995; 45(8):1561-9; PMID:7543987; <http://dx.doi.org/10.1212/WNL.45.8.1561>
71. De Servi B, La Porta CAM, Bontempelli M, Comolli R. Decrease of TGF-beta1 plasma levels and increase of nitric oxide synthase activity in leukocytes as potential biomarkers of Alzheimer's disease. *Exp. Gerontol.* 2002; 37(6):813-821; PMID:12175481; [http://dx.doi.org/10.1016/S0531-5565\(02\)00018-9](http://dx.doi.org/10.1016/S0531-5565(02)00018-9)
72. Tesseur I, Zou K, Esposito L, Bard F, Berber E, Can JV, Lin AH, Crews L, Tremblay P, Mathews P, et al. Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. *J. Clin. Invest.* 2006; 116(11):3060-9; PMID:17080199; <http://dx.doi.org/10.1172/JCI27341>

73. Mogi M, Harada M, Kondo T, Narabayashi H, Riederer P, Nagatsu T. Transforming growth factor- β 1 levels are elevated in the striatum and in ventricular cerebrospinal fluid in Parkinson's disease. *Neurosci. Lett.* 1995; 193(2):129-132; PMID:7478158; [http://dx.doi.org/10.1016/0304-3940\(95\)11686-Q](http://dx.doi.org/10.1016/0304-3940(95)11686-Q)
74. Qian L, Wei S-J, Zhang D, Hu X, Xu Z, Wilson B, El-Benna J, Hong JS, Flood PM. Potent Anti-Inflammatory and Neuroprotective Effects of TGF- β 1 Are Mediated through the Inhibition of ERK and p47phox-Ser345 Phosphorylation and Translocation in Microglia. *J. Immunol.* 2008; 181(1):660-668; PMID:18566433; <http://dx.doi.org/10.4049/jimmunol.181.1.660>
75. Nicoletti F, Di Marco R, Patti F, Reggio E, Nicoletti A, Zaccane P, Stivala F, Meroni PL, Reggio A. Blood levels of transforming growth factor-beta 1 (TGF-beta1) are elevated in both relapsing remitting and chronic progressive multiple sclerosis (MS) patients and are further augmented by treatment with interferon-beta 1b (IFN-beta1b). *Clin. Exp. Immunol.* 1998; 113(1):96-9; PMID:9697990; <http://dx.doi.org/10.1046/j.1365-2249.1998.00604.x>
76. Racke MK, Dhib-Jalbut S, Cannella B, Albert PS, Raine CS, McFarlin DE. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor beta 1. *J. Immunol.* 1991; 146(9):3012-3017; PMID:1707929
77. Liu A, Niswander LA. Bone morphogenetic protein signalling and vertebrate nervous system development. *Nat. Rev. Neurosci.* 2005; 6(12):945-54; PMID:16340955; <http://dx.doi.org/10.1038/nrn1805>
78. Xie Z, Sheng N, Jing N. BMP signaling pathway and spinal cord development. *Front. Biol. (Beijing).* 2012; 7(1):24-29
79. Araya R, Kudo M, Kawano M, Ishii K, Hashikawa T, Iwasato T, Itoharu S, Terasaki T, Oohira A, Mishina Y, et al. BMP signaling through BMPRIA in astrocytes is essential for proper cerebral angiogenesis and formation of the blood-brain-barrier. *Mol. Cell. Neurosci.* 2008; 38(3):417-30; PMID:18501628; <http://dx.doi.org/10.1016/j.mcn.2008.04.003>
80. Crews L, Adame A, Patrick C, Delaney A, Pham E, Rockenstein E, Hansen L, Masliah E. Increased BMP6 levels in the brains of Alzheimer's disease patients and APP transgenic mice are accompanied by impaired neurogenesis. *J. Neurosci.* 2010; 30(37):12252-62; PMID:20844121; <http://dx.doi.org/10.1523/JNEUROSCI.1305-10.2010>
81. Li D, Tang J, Xu H, Fan X, Bai Y, Yang L. Decreased hippocampal cell proliferation correlates with increased expression of BMP4 in the APPswe/PS1DeltaE9 mouse model of Alzheimer's disease. *Hippocampus* 2008; 18(7):692-8; PMID:18398851; <http://dx.doi.org/10.1002/hipo.20428>
82. Tang J, Song M, Wang Y, Fan X, Xu H, Bai Y. Noggin and BMP4 co-modulate adult hippocampal neurogenesis in the APP(swe)/PS1(DeltaE9) transgenic mouse model of Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 2009; 385(3):341-5; PMID:19463786; <http://dx.doi.org/10.1016/j.bbrc.2009.05.067>
83. Harvey BK, Mark A, Chou J, Chen GJ, Hoffer BJ, Wang Y. Neurotrophic effects of bone morphogenetic protein-7 in a rat model of Parkinson's disease. *Brain Res.* 2004; 1022(1-2):88-95; PMID:15353217; <http://dx.doi.org/10.1016/j.brainres.2004.06.072>
84. Stull ND, Jung JW, Iacovitti L. Induction of a dopaminergic phenotype in cultured striatal neurons by bone morphogenetic proteins. *Dev. Brain Res.* 2001; 130(1):91-98; [http://dx.doi.org/10.1016/S0165-3806\(01\)00216-4](http://dx.doi.org/10.1016/S0165-3806(01)00216-4)
85. Brederlau A. Bone Morphogenetic Proteins but Not Growth Differentiation Factors Induce Dopaminergic Differentiation in Mesencephalic Precursors. *Mol. Cell. Neurosci.* 2002; 21(3):367-378; PMID:12498780; <http://dx.doi.org/10.1006/mcne.2002.1178>
86. Ara J, See J, Mamontov P, Hahn A, Bannerman P, Pleasure D, Grinspan JB. Bone morphogenetic proteins 4, 6 and 7 are up-regulated in mouse spinal cord during experimental autoimmune encephalomyelitis. *J. Neurosci. Res.* 2008; 86(1):125-35; PMID:17722066; <http://dx.doi.org/10.1002/jnr.21462>
87. Deininger M, Meyermann R, Schluesener H. Detection of two transforming growth factor-beta-related morphogens, bone morphogenetic proteins-4 and -5, in RNA of multiple sclerosis and Creutzfeldt-Jakob disease lesions. *Acta Neuropathol.* 1995; 90(1):76-9; PMID:7572083; <http://dx.doi.org/10.1007/BF00294462>
88. Sabo JK, Aumann TD, Merlo D, Kilpatrick TJ, Cate HS. Remyelination is altered by bone morphogenic protein signaling in demyelinated lesions. *J. Neurosci.* 2011; 31(12):4504-10; PMID:21430151; <http://dx.doi.org/10.1523/JNEUROSCI.5859-10.2011>
89. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* 1996; 87(7):1171-80; PMID:8980224; [http://dx.doi.org/10.1016/S0092-8674\(00\)81813-9](http://dx.doi.org/10.1016/S0092-8674(00)81813-9)
90. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997; 277(5322):55-60; PMID:9204896; <http://dx.doi.org/10.1126/science.277.5322.55>
91. Lee HJ, Cho C-H, Hwang S-J, Choi HH, Kim KT, Ahn SY, Kim JH, Oh JL, Lee GM, Koh GY. Biological characterization of angiopoietin-3 and angiopoietin-4. *FASEB J.* 2004; 18(11):1200-8; PMID:15284220; <http://dx.doi.org/10.1096/fj.03.1466com>
92. Meng Z, Li M, He Q, Jiang S, Zhang X, Xiao J, Bai Y. Ectopic expression of human angiopoietin-1 promotes functional recovery and neurogenesis after focal cerebral ischemia. *Neuroscience* 2014; 267:135-46; PMID:24607344; <http://dx.doi.org/10.1016/j.neuroscience.2014.02.036>
93. Marteau L, Pacary E, Valable S, Bernaudin M, Guillemot F, Petit E. Angiopoietin-2 regulates cortical neurogenesis in the developing telencephalon. *Cereb. Cortex* 2011; 21(7):1695-702; <http://dx.doi.org/10.1093/cercor/bhq243>
94. Thurston G. Leakage-Resistant Blood Vessels in Mice Transgenically Overexpressing Angiopoietin-1. *Science* (80-.). 1999; 286(5449):2511-2514; <http://dx.doi.org/10.1126/science.286.5449.2511>
95. Thurston G, Rudge JS, Ioffe E, Zhou H, Ross L, Croll SD, Glazer N, Holash J, McDonald DM, Yancopoulos GD. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat. Med.* 2000; 6(4):460-3; PMID:10742156; <http://dx.doi.org/10.1038/74725>
96. Zhang ZG, Zhang L, Tsang W, Soltanian-Zadeh H, Morris D, Zhang R, Goussev A, Powers C, Yeich T, Chopp M. Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* 2002; 22(4):379-92; PMID:11919509; <http://dx.doi.org/10.1097/00004647-200204000-00002>
97. Nourhaghighi N, Teichert-Kuliszewska K, Davis J, Stewart DJ, Nag S. Altered Expression of Angiopoietins During Blood-Brain Barrier Breakdown and Angiogenesis. *Lab. Invest.* 2003; 83(8):1211-1222; <http://dx.doi.org/10.1097/01.LAB.0000082383.40635.FE>
98. Yu H, Wang P, An P, Xue Y, Yixue X. Recombinant human angiopoietin-1 ameliorates the expressions of ZO-1, occludin, VE-cadherin, and PKC α signaling after focal cerebral ischemia/reperfusion in rats. *J. Mol. Neurosci.* 2012; 46(1):236-47; PMID:21710361; <http://dx.doi.org/10.1007/s12031-011-9584-5>
99. Zhao Y, Li Z, Wang R, Wei J, Li G, Zhao H. Angiopoietin 1 counteracts vascular endothelial growth factor-induced blood-brain barrier permeability and alleviates ischemic injury in the early stages of transient focal cerebral ischemia in rats. *Neurol. Res.* 2010; 32(7):748-55; PMID:19660197; <http://dx.doi.org/10.1179/016164109X12445616596562>
100. Avraham HK, Jiang S, Fu Y, Nakshatri H, Ovadia H, Avraham S. Angiopoietin-2 mediates blood-brain barrier impairment and colonization of triple-negative breast cancer cells in brain. *J. Pathol.* 2014; 232(3):369-81; PMID:24421076; <http://dx.doi.org/10.1002/path.4304>
101. Lai D-M, Li H, Lee C-C, Tzeng YS, Hsieh YH, Hsu WM, Hsieh FJ, Cheng JT, Tu YK. Angiopoietin-like protein 1 decreases blood brain barrier damage and edema following focal cerebral ischemia in mice. *Neurochem. Int.* 2008; 52(3):470-7; PMID:17919782; <http://dx.doi.org/10.1016/j.neuint.2007.08.010>
102. Bouletti C, Mathivet T, Coqueran B, Serfaty JM, Lesage M, Berland E, Ardicie-Robouant C, Kauffenstein G, Henrion D, Lapergue B, et al. Protective effects of angiopoietin-like 4 on cerebrovascular and functional damages in ischaemic stroke. *Eur. Heart J.* 2013; 34(47):3657-68; PMID:23674618; <http://dx.doi.org/10.1093/eurheartj/ehi153>
103. Thirumangalakudi L, Samany PG, Owoso A, Wisakar B, Grammas P. Angiogenic proteins are expressed by brain blood vessels in Alzheimer's disease. *J. Alzheimers. Dis.* 2006; 10(1):111-8; PMID:16988487
104. Paris D, Townsend K, Quadros A, Humphrey J, Sun J, Brem S, Wotoczek-Obadia M, DelleDonne A, Patel N, Obregon DF, et al. Inhibition of angiogenesis by Abeta peptides. *Angiogenesis* 2004; 7(1):75-85; PMID:15302999; <http://dx.doi.org/10.1023/B:AGEN.0000037335.17717.bf>
105. Sillén A, Brohede J, Liljus L, Forsell C, Andrade J, Odeberg J, Ebise H, Winblad B, Graff C. Linkage to 20p13 including the ANGPT4 gene in families with mixed Alzheimer's disease and vascular dementia. *J. Hum. Genet.* 2010; 55(10):649-55; <http://dx.doi.org/10.1038/jhg.2010.79>
106. Jiang H, Zhang F, Yang J, Han S. Angiopoietin-1 ameliorates inflammation-induced vascular leakage and improves functional impairment in a rat model of acute experimental autoimmune encephalomyelitis. *Exp. Neurol.* 2014; 261:245-57; PMID:24852101; <http://dx.doi.org/10.1016/j.expneurol.2014.05.013>
107. Luo Y, Raible D, Raper JA. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* 1993; 75(2):217-27; PMID:8402908; [http://dx.doi.org/10.1016/0092-8674\(93\)80064-L](http://dx.doi.org/10.1016/0092-8674(93)80064-L)
108. Unified nomenclature for the semaphorins/collapsins. Semaphorin Nomenclature Committee. *Cell* 1999; 97(5):551-2; PMID:10367884; [http://dx.doi.org/10.1016/S0092-8674\(00\)80766-7](http://dx.doi.org/10.1016/S0092-8674(00)80766-7)
109. Tamagnone L, Artigiani S, Chen H, He Z, Ming GL, Song H, Chedotal A, Winberg ML, Goodman CS, Poo M, et al. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* 1999; 99(1):71-80; PMID:10520995; [http://dx.doi.org/10.1016/S0092-8674\(00\)80063-X](http://dx.doi.org/10.1016/S0092-8674(00)80063-X)
110. He Z, Tessier-Lavigne M. Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell* 1997; 90(4):739-51; PMID:9288753; [http://dx.doi.org/10.1016/S0092-8674\(00\)80534-6](http://dx.doi.org/10.1016/S0092-8674(00)80534-6)
111. Kconcina E, Roth L, Gonthier B, Bagnard D. Role of Semaphorins during Axon Growth and Guidance. *Adv Exp Med Biol* 621:50-64
112. Bagnard D, Lohrum M, Uziel D, Püschel AW, Bolz J. Semaphorins act as attractive and repulsive guidance signals during the development of cortical projections. *Development* 1998; 125(24):5043-53; PMID:9811588
113. De Castro F, Hu L, Drabkin H, Sotelo C, Chédotal A. Chemoattraction and chemorepulsion of olfactory bulb axons by different secreted semaphorins. *J. Neurosci.* 1999; 19(11):4428-36; PMID:10341244

114. Serini G, Valdembrì D, Zanivan S, Morterra G, Burkhart C, Caccavari F, Zammataro L, Primo L, Tamagnone L, Logan M, et al. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* 2003; 424(6947):391-7; PMID:12879061; <http://dx.doi.org/10.1038/nature01784>
115. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. *Nat. Immunol.* 2008; 9(1):17-23; PMID:18180379; <http://dx.doi.org/10.1038/ni1553>
116. Acevedo LM, Barillas S, Weis SM, Göthert JR, Cheresh DA. Semaphorin 3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor. *Blood* 2008; 111(5):2674-80; PMID:18180379; <http://dx.doi.org/10.1182/blood-2007-08-110205>
117. Le Guelte A, Galan-Moya E-M, Dwyer J, Treps L, Kertler G, Hebda JK, Dubois S, Auffray C, Chneiweiss H, Bidere N, et al. Semaphorin 3A elevates endothelial cell permeability through PP2A inactivation. *J. Cell Sci.* 2012; 125(Pt 17):4137-46; PMID:22685328; <http://dx.doi.org/10.1242/jcs.108282>
118. Smith ES, Jonason A, Reilly C, Veeraghavan J, Fisher T, Doherty M, Klimatcheva E, Mallow C, Cornelius C, Leonard JE, et al. SEMA4D compromises blood-brain barrier, activates microglia, and inhibits remyelination in neurodegenerative disease. *Neurobiol. Dis.* 2015; 73:254-268; <http://dx.doi.org/10.1016/j.nbd.2014.10.008>
119. Good PF, Alapat D, Hsu A, Chu C, Perl D, Wen X, Burstein DE, Kohtz DS. A role for semaphorin 3A signaling in the degeneration of hippocampal neurons during Alzheimer's disease. *J. Neurochem.* 2004; 91(3):716-36; PMID:15485501; <http://dx.doi.org/10.1111/j.1471-4159.2004.02766.x>
120. Jun G, Asai H, Zeldich E, Drapeau E, Chen C, Chung J, Park JH, Kim S, Haroutunian V, Foroud T, et al. PLXNA4 is associated with Alzheimer disease and modulates tau phosphorylation. *Ann. Neurol.* 2014; 76(3):379-92; PMID:25043464; <http://dx.doi.org/10.1002/ana.24219>
121. Clarimon J, Scholz S, Fung H-C, Hardy J, Eerola J, Hellstrom O, Chen CM, Wu YR, Tienari PJ, Singleton A. Conflicting results regarding the semaphorin gene (SEMA5A) and the risk for Parkinson disease. *Am. J. Hum. Genet.* 2006; 78(6):1082-4; author reply 1092-4; PMID:16685660; <http://dx.doi.org/10.1086/504727>
122. Okuno T, Nakatsuji Y, Kumanogoh A. The role of immune semaphorins in multiple sclerosis. *FEBS Lett.* 2011; 585(23):3829-35; PMID:21420960; <http://dx.doi.org/10.1016/j.febslet.2011.03.033>
123. Pardridge WM. Molecular Trojan horses for blood-brain barrier drug delivery. *Curr. Opin. Pharmacol.* 2006; 6(5):494-500; PMID:16839816; <http://dx.doi.org/10.1016/j.coph.2006.06.001>
124. Peluffo H, Unzueta U, Negro-Demontel ML, Xu Z, Vázquez E, Ferrer-Miralles N, Villaverde A. BBB-targeting, protein-based nanomedicines for drug and nucleic acid delivery to the CNS. *Biotechnol. Adv.* 33(2):277-87; PMID:25698504; <http://dx.doi.org/10.1016/j.biotechadv.2015.02.004>
125. Southwell AL, Franciosi S, Villanueva EB, Xie Y, Winter LA, Veeraghavan J, Jonason A, Felczak B, Zhang W, Kovalik V, et al. Anti-semaphorin 4D immunotherapy ameliorates neuropathology and some cognitive impairment in the YAC128 mouse model of Huntington disease. *Neurobiol. Dis.* 2015; 76:46-56; PMID:25662335; <http://dx.doi.org/10.1016/j.nbd.2015.01.002>
126. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; 425(6958):577-84; PMID:14534577; <http://dx.doi.org/10.1038/nature02006>
127. Pendaries V, Verrecchia F, Michel S, Mauviel A. Retinoic acid receptors interfere with the TGF-beta/Smad signaling pathway in a ligand-specific manner. *Oncogene* 2003; 22(50):8212-20; PMID:14603262; <http://dx.doi.org/10.1038/sj.onc.1206913>
128. Sheng N, Xie Z, Wang C, Bai G, Zhang K, Zhu Q, Song J, Guillemot F, Chen YG, Lin A, et al. Retinoic acid regulates bone morphogenetic protein signal duration by promoting the degradation of phosphorylated Smad1. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107(44):18886-91; PMID:20956305; <http://dx.doi.org/10.1073/pnas.1009244107>
129. Guo X, Wang X-F. Signaling cross-talk between TGF-beta/BMP and other pathways. *Cell Res.* 2009; 19(1):71-88; PMID:19002158; <http://dx.doi.org/10.1038/cr.2008.302>
130. Kim W, Moon S-O, Lee SY, Jang KY, Cho CH, Koh GY, Choi KS, Yoon KH, Sung MJ, Kim DH, et al. COMP-angiopoietin-1 ameliorates renal fibrosis in a unilateral ureteral obstruction model. *J. Am. Soc. Nephrol.* 2006; 17(9):2474-83; PMID:16885409; <http://dx.doi.org/10.1681/ASN.2006020109>
131. Kim W. The role of angiopoietin-1 in kidney disease. *Electrolyte Blood Press.* 2008; 6(1):22-6.
132. Nakamura T, Okada T, Endo M, Kadomatsu T, Taniwaki T, Sei A, Odagiri H, Masuda T, Fujimoto T, Nakamura T, et al. Angiopoietin-like protein 2 induced by mechanical stress accelerates degeneration and hypertrophy of the ligamentum flavum in lumbar spinal canal stenosis. *PLoS One* 2014; 9(1):e85542; PMID:24465594; <http://dx.doi.org/10.1371/journal.pone.0085542>
133. Glinka Y, Prud'homme GJ. Neuropilin-1 is a receptor for transforming growth factor beta-1, activates its latent form, and promotes regulatory T cell activity. *J. Leukoc. Biol.* 2008; 84(1):302-10; PMID:18436584; <http://dx.doi.org/10.1189/jlb.0208090>
134. Cao Y, Szabolcs A, Dutta SK, Yaqoob U, Jagavelu K, Wang L, Leaf EB, Urrutia RA, Shah VH, Mukhopadhyay D. Neuropilin-1 mediates divergent R-Smad signaling and the myofibroblast phenotype. *J. Biol. Chem.* 2010; 285(41):31840-8; PMID:20675371; <http://dx.doi.org/10.1074/jbc.M110.151696>
135. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res.* 2009; 19(1):128-39; PMID:19114990; <http://dx.doi.org/10.1038/cr.2008.328>
136. Yen A, Roberson MS, Varvayanis S, Lee AT. Retinoic acid induced mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase-dependent MAP kinase activation needed to elicit HL-60 cell differentiation and growth arrest. *Cancer Res.* 1998; 58(14):3163-72; PMID:9679985
137. Bikkavilli RK, Malbon CC. Mitogen-activated protein kinases and Wnt/beta-catenin signaling: Molecular conversations among signaling pathways. *Commun. Integr. Biol.* 2009; 2(1):46-9; PMID:19513264; <http://dx.doi.org/10.4161/cib.2.1.7503>
138. Guardavaccaro D, Clevers H. Wnt/beta-catenin and MAPK signaling: allies and enemies in different battlefields. *Sci. Signal.* 2012; 5(219):pe15; PMID:22494969; <http://dx.doi.org/10.1126/scisignal.2002921>
139. Elia D, Madhala D, Ardon E, Reshef R, Halevy O. Sonic hedgehog promotes proliferation and differentiation of adult muscle cells: Involvement of MAPK/ERK and PI3K/Akt pathways. *Biochim. Biophys. Acta* 2007; 1773(9):1438-46
140. Kessaris N, Jämen F, Rubin LL, Richardson WD. Cooperation between sonic hedgehog and fibroblast growth factor/MAPK signalling pathways in neocortical precursors. *Development* 2004; 131(6):1289-98; PMID:14960493; <http://dx.doi.org/10.1242/dev.01027>
141. Zhu W-H, MacIntyre A, Nicosia RF. Regulation of angiogenesis by vascular endothelial growth factor and angiopoietin-1 in the rat aorta model: distinct temporal patterns of intracellular signaling correlate with induction of angiogenic sprouting. *Am. J. Pathol.* 2002; 161(3):823-30; PMID:12213710; [http://dx.doi.org/10.1016/S0002-9440\(10\)64242-3](http://dx.doi.org/10.1016/S0002-9440(10)64242-3)
142. Hashiramoto A, Sakai C, Yoshida K, Tsumiyama K, Miura Y, Shiozawa K, Nose M, Komai K, Shiozawa S. Angiopoietin 1 directly induces destruction of the rheumatoid joint by cooperative, but independent, signaling via ERK/MAPK and phosphatidylinositol 3-kinase/Akt. *Arthritis Rheum.* 2007; 56(7):2170-9; PMID:17599743; <http://dx.doi.org/10.1002/art.22727>
143. Li Y, Chen J, Wu C, Wang L, Lu M, Chen X. Hepatitis B virus/hepatitis C virus upregulate angiopoietin-2 expression through mitogen-activated protein kinase pathway. *Hepatol. Res.* 2010; 40(10):1022-33; PMID:20887338; <http://dx.doi.org/10.1111/j.1872-034X.2010.00712.x>
144. He D-K, Shao Y-R, Zhang L, Shen J, Zhong ZY, Wang J, Xu G. Adenovirus-delivered angiopoietin-1 suppresses NF-kB and p38 MAPK and attenuates inflammatory responses in phosgene-induced acute lung injury. *Inhal. Toxicol.* 2014; 26(3):185-92; PMID:24517841; <http://dx.doi.org/10.3109/08958378.2013.872213>
145. Aurandt J, Li W, Guan K-L. Semaphorin 4D activates the MAPK pathway downstream of plexin-B1. *Biochem. J.* 2006; 394(Pt 2):459-64; PMID:16187944; <http://dx.doi.org/10.1042/BJ20051123>
146. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* 2003; 424(6947):398-405; PMID:12879062; <http://dx.doi.org/10.1038/nature01790>
147. López-Carballo G, Moreno L, Masía S, Pérez P, Barettino D. Activation of the phosphatidylinositol 3-kinase/Akt signaling pathway by retinoic acid is required for neural differentiation of SH-SY5Y human neuroblastoma cells. *J. Biol. Chem.* 2002; 277(28):25297-304; <http://dx.doi.org/10.1074/jbc.M201869200>
148. Ohashi E, Kogai T, Kagechika H, Brent GA. Activation of the PI3 kinase pathway by retinoic acid mediates sodium/iodide symporter induction and iodide transport in MCF-7 breast cancer cells. *Cancer Res.* 2009; 69(8):3443-50; PMID:19351850; <http://dx.doi.org/10.1158/0008-5472.CAN-08-3234>
149. Rooff C, Krohn S, Kretschmar C, et al. PI3K/Akt Signaling Interacts With Wnt/beta-Catenin Signaling But Does Not Induce An Accumulation Of beta-Catenin In The Nucleus Of Acute Lymphoblastic Leukemia Cell Lines. *Blood* 2013; 122(21):4886
150. Riobó NA, Lu K, Ai X, Haines GM, Emerson CP. Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103(12):4505-10; <http://dx.doi.org/10.1073/pnas.0504337103>
151. Kim I, Kim HG, So JN, Kim JH, Kwak HJ, Koh GY. Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. *Circ. Res.* 86(1):24-9; PMID:10625301; <http://dx.doi.org/10.1161/01.RES.86.1.24>
152. Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. *Mol. Cell. Biol.* 2009; 29(8):2011-22; PMID:19223473; <http://dx.doi.org/10.1128/MCB.01472-08>
153. Pasterkamp RJ. Semaphorins: receptor and intracellular mechanisms. *Adv. Exp. Med. Biol.* 2007; 600:38-51; PMID:17607945; <http://dx.doi.org/10.1007/978-0-387-70956-7>